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# THE EXAMINATION OF MILK
FOR
# PUBLIC HEALTH PURPOSES

JOSEPH RACE, F.I.C.

*City Bacteriologist and Food Examiner, Ottawa; Chairman of Committee on Standard Methods of Analysis, Canadian Public Health Association, Member of Committee on Municipal Food Administration, American Public Health Association*

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# PREFACE

This volume is primarily intended as a practical handbook for those engaged in the chemical and bacteriological examination of milk for public health purposes, but it is also hoped that it will be of material assistance to students and others who have previously assimilated the fundamentals of bacteriological technique.

The control of milk supplies was formerly confined to a chemical examination for adulteration, but since the beginning of the 20th century the bacteriological examination has been regarded as a "sine qua non," and in America the present tendency is to have both examinations made under the supervision of the Public Health Authorities. For this reason no apology is necessary for the inclusion of chemical methods and the data which will enable the examiner to interpret the results obtained.

In the bacteriological section an attempt has been made to include all methods that have been proved to be reliable and in some instances the details of the standard methods of the American Public Health Association have been given; in other cases the report as published by the A.P.H.A. should be consulted.

The tables of bacteriological results have been added in the hope that they will lead to the standardisation of records. At present the results reported by many laboratories are not comparable because of the form in which they are issued.

Joseph Race.

Ottawa, Ont.,
December, 1917.



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# EXAMINATION OF MILK FOR PUBLIC HEALTH PURPOSES

## CHAPTER I

### CONSTITUENTS OF MILK

Milk is the opaque white fluid which is secreted by the mammary glands. It consists essentially of an emulsion of fat and a colloidal solution of caseinogen in water containing lactose and traces of mineral matter.

**Milk fat,** with which is associated small quantities of cholesterol, lecithin, and a trace of colouring matter, consists of a mixture of triglycerides of various fatty acids. These acids are mixtures of the straight chain series $C_nH_{2n+1}COOH$ and $C_nH_{2n-1}COOH$, the less saturated acids being, according to the best information, entirely absent. The relative proportions of the various acids are by no means constant, being dependent upon various factors such as foodstuffs, seasonal variations, breed of cattle, and climatic conditions.

The fat is present in milk as enormous numbers of very small globules and it is the reflection of light from these particles and those of caseinogen that produces the characteristic white opaque appearance of milk. Although it was formerly held that the fat globules were surrounded by albuminous membranes which preserved the form, it is now generally accepted that this is due to surface tension and that the size of the globules can be altered by physical methods.

The size of the fat globules in milk varies from $0.8\mu$ to $20\mu$ with an average of about $2.7\mu$ and the number of globules from $19\times10^8$ to $60\times10^8$ per cubic centimeter. Although no

definite relation has been established between the breed of cattle and the size and number of globules there are a number of results which indicate that during interrupted milking the size of the globules increases with the fat content and also that as the lactation period proceeds the globules decrease in size and increase in number (see p. 43).

The origin and method of formation of milk fat have not been discovered although many hypotheses have been proposed. The normal process seems to be the formation of milk fat, directly or indirectly, from nutritive fat, but when this source is eliminated the formation of milk fat proceeds, though diminished in activity, by drawing upon the body fat. Even when the body fat is exhausted, milk fat can be formed: this is attributed to proteids acting as the source of fat.

The various analytical and physical constants of milk fat are:

| | |
|---|---|
| Specific gravity $\frac{37.8}{37.8}$ | 0.9094–0.9140 |
| Refractive index, 35° C | 1.4550–1.4586 |
| Melting-point | 28° C.–36° C. |
| Solidifying point | 21° C.–27° C. |
| Reichert-Wollny value | 25–27 |
| Iodine absorption | 31–35 |

The calorific value of butter fat, according to Stohmann, is 9.231 calories per gram and according to Atwater, from 9.320 to 9.362 calories. A value of 9.3 is usually employed in calculating the calorific value of milk fat. The molecular weight of fat, as calculated from the amount of alkali required for saponification and assuming that all the acids present are monobasic, is from 720-740, whilst the direct determination by the cryoscopic method points to values from 696–716. The presence of dibasic acids would harmonise these two sets of results, but such acids have not been isolated from butter fat.

**Lactose.** Although there is some evidence of the presence of traces of a monosaccharide in milk, the carbohydrate secreted

under normal conditions is lactose or milk sugar. Lactose is a disaccharide of the empirical formula $C_{12}H_{22}O_{11}$ and is found in the milk of most mammals. Lactose is secreted in the gland and is found only in the milk, though, if suckling is interrupted, it may appear in the urine, from which is it eliminated on removal of the lactating gland: if the gland is removed before the lactation period commences it may not appear at all. The fact that the blood in the mammary vein before parturition and during lactation contains less dextrose than the blood of the jugular vein (Kaufman and Lagne) suggests either dextrose, or the constituents from which dextrose is formed, as the source of lactose.

Two forms of lactose exist and are known as the alpha and beta varieties. When lactose is obtained by crystallisation from water, the alpha modification, which crystallises in the rhombic form, is formed: this modification exhibits the phenomenon of multirotation, i.e., shows a decreasing specific rotation with lapse of time after solution in water. For a short period of time, the length of which depends upon the temperature, the solution of alpha lactose shows a specific rotation of $[\alpha]_D = +84.0$, but this gradually diminishes until a value of $+52.5$ is reached, this being the specific rotation of the stable variety of lactose containing one molecule of water. The corresponding value of the anhydrous lactose is $+55.3$. Anhydrous lactose, obtained by heating the hydrated carbohydrate to 130° C., does not produce multi-rotation in aqueous solutions. The beta modification, produced by rapid evaporation of aqueous solutions of lactose in metal vessels, has a specific rotation $[\alpha]_D + 32.7$ and shows the same birotation ratio, i.e., $\frac{\text{initial rotation}}{\text{final rotation}}$ as the alpha modification, viz., 1.6. This shows that the reaction is mono-molecular in character. The density of the alpha variety is 1.545 $\frac{15.5}{15.5}$ and that of a solution containing 10 grams per 100 c.cms., 1.0391 $\frac{15.5}{15.5}$. The specific rotation is $[\alpha]_D = 52.5$ at 20° C. and is lowered 0.075 for each degree rise in temperature. The refractive index $\mu_D^{20°}$ of

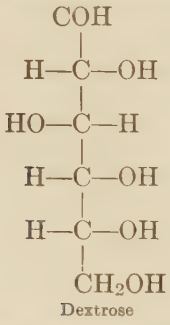
a solution containing 10 grams per 100 c.cms. is 1.3461 and of a 5 per cent solution 1.3395.

Lactose is not fermented by ordinary yeast (Saccharomycetes cerevisiæ) and is not affected by the ordinary enzymes. The enzyme lactase, which is capable of hydrolysing lactose into dextrose and galactose, is found as an endo enzyme in Torula kefyr and T. tyrcola and also as an exo enzyme in Kefyr grains.

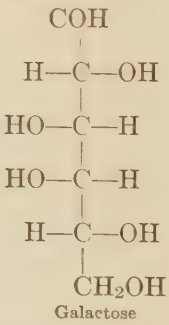
$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Galactose}}{C_6H_{12}O_6}.$$

Lactase is also widely distributed in the animal kingdom, being present in the mucous membrane of the stomachs of infants and also in the expressed juices of muscle, liver, lungs, and pancreas.

The action of acids generally is similar to that of lactase, though the mineral acids are much more effective than those of the organic series. Dextrose and galactose, according to Fischer, have the following constitutional formulæ:



Dextrose

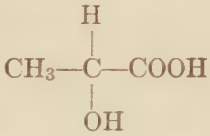


Galactose

These formulæ show both sugars to be isomeric aldoses of the monose type. Their specific rotatory powers $[\alpha]_D$ are

| | Dextrose. | Galactose. |
|---|---|---|
| Equilibrium form | 52.7 | 80.3 |
| Alpha modification | 105. | 120. |
| Birotation ratio | 2. | 1.5 |

The most important products derived from lactose, in connection with the bacteriological examination of milk, are the lactic acids. Lactic acid ($C_3H_6O_3$) exists as four different isomers, three having the constitutional formula $CH_3 \cdot CH(OH) \cdot COOH$ or alpha hydroxy propionic acid, and one $CH_2(OH) \cdot CH_2 \cdot COOH$ hydracrylic acid or beta hydroxy propionic acid. As the latter is not produced during the bacterial decomposition of lactose no further description of this acid is necessary in this work. Alpha hydroxy propionic acid, or lactic acid as it is usually known as, contains an asymmetric carbon atom



and exists, therefore, in three different forms, viz., dextro, lævo, and racemic or inactive lactic acids. The dextro and lævo rotatory acids are both produced by micro-organisms, but unless pure cultures are employed the majority of the acid produced is of the racemic ($d+l$) variety.

The density of lactic acid is $1.2485\ {}^{15^\circ}_{4^\circ}$ and the refractive index $\mu_D 20^\circ$ 1.4469. On evaporation of aqueous solutions of lactic acid dehydrolactic acid $C_6H_{10}O_5$ is produced, and, ultimately, at higher temperatures, lactide $C_6H_8O_4$ is formed. The boiling point of lactic acid is 83° C. at 1 mm. pressure and 119° C. at 12 mm. pressure. Lactic acid, though insoluble in petroleum ether, is soluble in, and miscible with alcohol and ether in all proportions.

Lactic acid forms well-defined salts with various metals and these may be used for the separation of the acid. The calcium salt which crystallises with 5 molecules of water is soluble to the extent of 9.5 per cent in cold water: zinc lactate ($ZnC_6H_{10}O_4 \cdot 3H_2O$) is less soluble, 1.3 per cent in cold water and 13 per cent in hot, and forms well-defined monoclinic prisms.

**Proteids.** The proteids of milk are:

| | Per Cent. |
|---|---|
| Caseinogen | approximately 2.0–3.0 |
| Lactalbumin | approximately 0.3–0.8 |
| Lactoglobulin | a trace |
| Mucoid proteid | a trace |

Caseinogen* is a distinctly acid phospho proteid which does not contain purine or pyrimidine derivatives. Lactalbumin, as its name implies, is one of the albumins and, therefore, soluble in water and coagulated by heat. Lactoglobulin is insoluble in water but soluble in salt solutions.

According to Richmond the proteids of milk are characterised by the following reactions: Caseinogen is precipitated by adding sodium chloride, magnesium sulphate, or ammonium sulphate to saturation: globulin is soluble in a saturated solution of sodium chloride but is precipitated by magnesium and ammonium sulphates: albumin is soluble in saturated solutions of sodium chloride and magnesium sulphate but is precipitated by ammonium sulphate. Albumin, however, may be precipitated by magnesium sulphate in slightly acid solutions but is redissolved on neutralisation of the solution. These reactions are relative rather than specific and cannot be relied upon for quantitative separation of the various proteids: they may, however, be used for preparing the pure proteids by redissolving and reprecipitating the various fractions. Other methods may also be used for the separation of the proteids. For example, the caseinogen may be removed by the action of chymase, the lab ferment of rennet, or by filtration through coarse porcelain: filtration through fine porcelain or boiling with a small quantity of acid followed by filtration will remove all the proteids. Lactalbumin is slowly coagulated by heating at 70° C., but very little is precipitated when the acidity is normal. Casein-

* Caseinogen is used in these pages to designate the mother substance and paracasein the rennet transformation product: this nomenclature, though not strictly logical, eliminates the ambiguity that arises from the difference in the prevailing English and American phraseology.

ogen and albumin may also be precipitated by the addition of a solution of calcium chloride if the milk is previously heated to 35° to 45° C. All three proteids are soluble in alkalies and insoluble in alcohol and ether: their copper, mercury, and other salts of the heavy metals are insoluble, and all the lacto proteids are completely precipitated by tannin and phosphotungstic acids.

**Caseinogen,** when pure, is a white, amorphous, odourless, and tasteless substance which is practically insoluble in water. The specific gravity is 1.257. Owing to the stability of the additive compound which calcium caseinogenate forms with calcium phosphate, in which form it is present in milk, the preparation of pure caseinogen is a matter of considerable difficulty, and it is probable that at least a portion of the differences in composition found by various observers is due to this factor. Repeated precipitation and solution remove the greater part of the calcium but the last traces are extremely difficult to eliminate (Van Slyke and Bosworth[1]). Caseinogen is easily precipitated by the addition of a few drops of glacial acetic acid to milk diluted with an equal volume of water, and the precipitate may be redissolved by the addition of caustic alkalies, alkaline earths, ammonia, carbonates, bicarbonates, or phosphates, even in minute quantities. Schryver[2] has shown that if the caseinogen produced by precipitation with acetic acid is allowed to remain in contact with the excess of acid (1 in 1000) at room temperature, or is heated with water to 37° C., a product is formed the solubility of which in lime water is only about one-third that of natural caseinogen. This has been designated as "metacaseinogen," the solution of which in half saturated lime water is opalescent but not opaque. Metacaseinogen can be reconverted into caseinogen by solution in sodium hydrate and precipitation with acetic acid providing that the contact with the acid is not unduly prolonged. Metacaseinogen is identical in composition with caseinogen: the following are some of the more authentic analyses of caseinogen.

Most of the analyses given were obtained from material prepared by Hammerstein's method, i.e., by repeated precip-

TABLE I

| | C | H | O | N | S | P |
|---|---|---|---|---|---|---|
| Hammerstein (1883–1885) | 52.96 | 7.05 | 22.73 | 15.65 | 0.76 | 0.85 |
| Chittenden and Painter (1887) | 53.30 | 7.07 | 22.03 | 15.91 | 0.82 | 0.87 |
| Lehmann and Hempel (1894) | 54.00 | 7.04 | ..... | 15.60 | 0.77 | 0.85 |
| Ellenberger (1902) | 53.07 | 7.13 | 21.74 | 15.64 | 0.76 | 0.80 |
| Lacqueur and Sackur (1903) | ..... | ..... | ..... | 15.45 | 0.76 | 0.77 |
| Burow (1905) | 52.82 | 7.09 | 22.92 | 15.64 | 0.72 | 0.81 |
| Tangl (1908) | 52.69 | 6.81 | 23.14 | 15.65 | 0.83 | 0.88 |
| Van Slyke and Bosworth (1913) mean | 53.17 | 7.09 | 22.48 | 15.67 | 0.77 | 0.82 |
| Geake (1913) | 53.20 | 7.09 | 22.34 | 15.63 | 1.015 | 0.73 |

itation with acid and solution in alkali, and it is possible that during this process a portion of the sulphur was removed as sulphides as the sulphur portion of the molecule is slightly unstable. Lehmann's material was obtained by filtration through porous plates and probably contained a portion of the lime salts which constitute part of the caseinogen complex in milk. From the percentage composition, Richmond has calculated the empirical formula for caseinogen to be $C_{162}H_{258}N_{41}SPO_{52}$, and in support of this he quotes experiments [3] in which he found that $\frac{N}{100}$ potassium and sodium carbonate solutions, when treated with an excess of caseinogen, dissolved 1.83 and 1.86 parts per 100 c.cms., respectively. The above formula, according to Richmond, would give 1.84 parts per 100 c.cms. The author in some unpublished experiments, determined the solubility of caseinogen in $\frac{N}{100}$ KOH and obtained a value of 1.83 grams per 100 c.cms. at room temperature (67° F.): other temperatures, however, gave different values, so that these results cannot be regarded as having any bearing on the constitution or weight of the molecule. Various compounds of caseinogen with bases have been reported. Soldner [4] separated compounds of caseinogen and lime containing 1.11 and 1.67 per cent of Ca., re-

spectively. Lehmann's material as separated by filtration contained 1.02 to 1.25 per cent of Ca. Van Slyke and Bosworth[1] report four compounds with lime, containing 0.22, 0.44, 1.07, and 1.78 per cent of Ca. They also prepared compounds with ammonia, sodium, and potassium, containing 0.20 per cent $NH_4$, 0.26 per cent Na, and 0.44 per cent K.

The acidity of caseinogen has been determined by many observers with fairly good agreement. The more important results are:

| | 1 c.c. $\frac{N}{10}$NaOH equals | 1 gram Caseinogen equals |
|---|---|---|
| Lacqueur and Sackur | 0.1138 gr. caseinogen | 8.81 c.c. $\frac{N}{10}$NaOH |
| Mathaiopoulos | 0.11315 | 8.84 |
| Long | 0.1124 | 8.90 |
| Van Slyke and Bosworth | 0.1111 | 9.00 |

From the analysis of the lime salts, Van Slyke and Bosworth regard caseinogen as an octobasic acid and classify these salts as follows:

| Grams per 100 Grams Caseinogen. | | Name of Compound. | Reaction to | | Valencies Satisfied. |
|---|---|---|---|---|---|
| Ca | CaO | | Litmus. | Phenol Phthalein. | |
| 0.22 | 0.31 | Monocalcium caseinogenate | ..... | ..... | 1 |
| 0.44 | 0.62 | Di calcium caseinogenate | ..... | ..... | 2 |
| 1.07 | 1.50 | Neutral calcium caseinogenate | Neutral | Acid | 5 |
| 1.78 | 2.50 | Basic calcium caseinogenate | ..... | Neutral | 8 |

From a consideration of the dissociation values of caseinogenates in dilute solutions, Lacqueur and Sackur[5] regarded caseinogen as either a penta or hexabasic acid but a later investigation of the physical properties by Robertson[6] shows that it is

octobasic. This would give a molecular weight of approximately 8900.

Caseinogen, when dissolved in dilute alkali, has a pronounced lævo rotatory action on polarized light, but the specific rotation is not constant, varying from $-94.8$ to $-111.8$, according to the concentration and nature of the alkali used as the solvent (Long). The soluble salts of caseinogen may be divided into two classes (1) salts of the alkaline earths, and (2) salts of the alkalies. According to Osborne[7] these are distinguished by the inability of the former to pass through the film of the Martin gelatin filter and by the formation of opalescent solutions. The solutions of the second class filter through gelatin membranes and are translucent. Both classes of salts are neutral to phenolphthalein when the valency of caseinogen is entirely satisfied, but when litmus is used as the indicator no definite change is indicated and the point of neutralisation varies with the concentration of the solution (Schryver). Salts of copper, mercury, and lead, precipitate caseinogen from neutral solutions, and mercury salts are also effective in the presence of acid: the precipitates so obtained are not constant in composition but vary with the conditions obtaining. The insolubility of the compounds with the heavy metals is utilised in milk analysis in the preparation of protein free milk serum for use in the polarimeter and refractometer. Caseinogen also exhibits basic properties and combines with acids with the formation of clear solutions. Long[8] found that 1 gram of caseinogen combined with about 7 c.cms. of $\frac{N}{10}$ acid in the form of sulphuric, hydrochloric, hydrobromic, hydriodic, and acetic acids to form soluble salt like compounds. Some observers have stated that precipitated caseinogen also combined with acids but L. L. Van Slyke and D. D. Van Slyke[9] have shown that the observed loss of acid on precipitation was due to surface adsorption and depended upon the nature and concentration of the acid, the temperature, the duration of contact, and the degree of agitation.

When caseinogen is acted upon by formaldehyde, the amino groups condense with the H·CHO to form methylene derivatives. The resultant compounds are not digested by trypsin but can be decomposed by steam and the formaldehyde quantitatively recovered in the distillate. On the formation of methylene derivatives, the alkalinity due to amino groups disappears, and the caseinogen salt, which before condensation reacted neutral to phenolphthalein, becomes acid and can be quantitatively titrated with alkalies. This reaction is the basis of the aldehyde value (vide p. 75).

Caseinogen, on hydrolysis by pepsin, trypsin, or dilute acids, undergoes proteoclastic digestion with the formation of caseinogen proteoses or caseoses, as they have been called, which are soluble in water. These caseoses have been subdivided into proto and deutero caseoses by their solubility in ammonium sulphate solutions of certain concentration.

The ultimate products of the hydrolysis of caseinogen have been extensively investigated and the results of various observers, obtained with caseinogen from various sources, are given in Table II.

Caseinogen exists in milk as a salt combined with phosphate of calcium, and although the composition of this complex has been investigated by many chemists during the last sixty years, it is impossible even yet to state that it is definitely established. Richmond, from an analysis of the material separated by filtration through a porous cell, assumes that caseinogen exists in milk as a double calcium sodium caseinogenate combined with half a molecule of tricalcic phosphate. $C_{162}H_{255}N_{41}SPO_{52}\cdot Ca\cdot Na\frac{1}{2}(Ca_3P_2O_8)$. The quantity of acid required for the displacement of the sodium atom in this formula by hydrogen, would be 8.3 c.cms. of normal acid per litre of milk, and Richmond found that on adding 8.6 c.cms. N. hydrochloric acid or sulphuric acid, the caseinogen was precipitated on boiling, and that the acidity of the serum was equal to that of the milk after boiling. L. L. Van Slyke and Bosworth[10] have pointed out that deductions based on the acidity of milk

TABLE II

CASEINOGEN HYDROLYSIS PRODUCTS

| Products of Hydrolysis. | Cow's Milk. (Abderhalden, Fischer, Osborne and Greed, Morner, Fischer and Abderhalden, Hart.) | Goat's Milk. (Abderhalden and Schittenhelm.) | Human Milk. (Abderhalden and Schittenhelm.) |
|---|---|---|---|
| Glycine | 0 | 0 | |
| Alanine | 0.90 | 1.50 | |
| Valine | 1.00 | | |
| Leucine | 10.50 | 7.40 | |
| Phenylalanine | 3.20 | 2.75 | |
| Tyrosine | 4.50 | 4.95 | 4.71 |
| Serine | 0.45 | | |
| Cystine | 0.06 | | |
| Proline | 6.70 | 4.60 | |
| Oxyproline | 1.50 | | |
| Aspartic acid | 1.20 | 1.20 | |
| Glutamic acid | 11.00 | 12.00 | |
| Tryptophane | 1.50 | | |
| Arganine | 4.84 | | |
| Lysine | 5.80 | | |
| Histidine | 2.59 | | |
| Diaminotrioxy-dodecanic acid | 0.75 | | |
| Aminovaleric acid | 7.20 | | |
| Ammonia | 1.60 | | |

and milk serum, as determined in the usual way by direct titration with alkali, may be entirely fallacious because of the errors introduced by titrating phosphoric acid in the presence of lime salts. Cameron and Hurst[11] have shown that the following reactions may occur.

$$(1)\ CaHPO_4 + 2H_2O = Ca(OH)_2 + H_3PO_4.$$

$$(2)\ 2CaHPO_4 + Ca(OH)_2 = Ca_3P_2O_8 + 2H_2O.$$

These result in the presence of free phosphoric acid in place of neutral dicalcium phosphate and the acidity is, therefore,

apparently higher. When milk is filtered through porcelain, the acidity of the serum is usually approximately half that of the original milk on direct titration with alkali, but Van Slyke and Bosworth have shown that, if before determining the acidity, the lime salts are previously removed by precipitation with neutral potassium oxalate, the acidity of the serum is equal to that of the milk: in other words, the caseinogen calcium phosphate complex in milk is not acid to phenolphthalein but neutral. Van Slyke and Bosworth [12] filtered milk through porcelain but instead of analysing the precipitate, compared the serum and the original milk. This eliminates errors caused by the absorption of soluble salts if the first filtrates of serum are rejected. Their results show that caseinogen exists in milk as neutral calcium caseinogenate (caseinogen, $Ca_4$) and neutral dicalcium phosphate. These are not in chemical combination as they could be almost completely separated by mechanical methods.

The reaction of caseinogen with rennin, a lab ferment, is of considerable importance because of the information it yields regarding the constitution of caseinogen, and also on account of the presence of this ferment in the mucous lining of calves' stomachs and the similarity of its action to that of the gastric juices of the human stomach. Although this reaction has been the subject of probably more investigations than any other subject in biological chemistry the modus operandi and the nature of the reaction products are still comparatively obscure.

It has long been known that fresh milk coagulates in the stomachs of the higher animals. An aqueous extract of the inner lining of the stomach of the calf causes curdling and clots milk producing a semi-solid mass. These facts have been utilised since an early date in the manufacture of cheese.

The earlier views concerning the nature of this change need not be considered in detail as they have since been proved to be entirely erroneous. The one most commonly accepted regarded the action as one of decomposition of the milk sugar into acids, which directly or indirectly produced the phenomenon observed. The first important advance was made

when Heintz [13] found that the muscosa extract of stomachs possessed the property of clotting milk of an alkaline reaction. Hammerstein,[14] and Schmidt,[15] first showed that the coagulation of milk by rennin was due to a soluble ferment which was named "labferment" or "chymosin." Hammerstein thoroughly investigated the nature of the reaction and his conclusions met with fairly general acceptance until a few years ago. He showed that caseinogen was not in true solution in milk but in a state of colloidal suspension, and that the presence of a certain quantity of calcium phosphate was necessary for the reaction to occur: also that during the reaction the caseinogen was so altered that it was unable to remain in colloidal suspension and was precipitated in the presence of calcium phosphate as paracasein calcium phosphate. He further found that the caseinogen was split into at least two other proteids, casein (der Kase) better described as paracasein, and whey proteid (Molkeneiweiss). These were distinguished by the insolubility in water of the calcium salts of the former compared with the smaller molecule of the latter and the solubility of its calcium salts. The composition of these proteins according to Koster is shown in Table III.

TABLE III

| | Paracasein. | Whey Proteid. |
|---|---|---|
| Carbon | 52.88 | 50.33 |
| Hydrogen | 7.00 | 7.00 |
| Nitrogen | 15.84 | 13.25 |
| Phosphorous (Richmond) | 0.99 | |

From these figures Richmond has calculated the approximate formulæ for these substances to be.

Paracasein $C_{140}H_{222}N_{36}PO_{44}$.
Whey proteid $C_{22}H_{37}N_5O_{10}$.

Hammerstein concluded that the conversion of caseinogen into

paracasein was independent of the calcium salts present and this has been confirmed by later observers. Some chemists (Loevenhart[16] and Briot[17]), have claimed that an essential part of the rennin reaction is a modification of the mineral constituents, but Harden and Macallum[18] have recently shown that if caseinogen solutions are treated with sufficient rennin (1 : 1000) no addition of calcium salts is required: Schryver[2] found that clot formation could be obtained in the entire absence of calcium ions. Duclaux[19] was the first to find that no proteoclastic cleavage is produced by the action of rennin and this has been confirmed by Van Slyke and Bosworth,[20] Geake,[21] and Harden and Macallum.[18] Loevenhart[16] suggested that caseinogen and paracasein were chemically identical and that the differences in behaviour were due to changes in molecular association or aggregation. This view is supported by Van Slyke and Hart[22] and Van Slyke and Bosworth (vide supra) who suggested that calcium caseinogenate is split by the action of rennin into two molecules of calcium paracaseinate which is identical in percentage composition with the original substance. Liwschiz[23] attempted to differentiate caseinogen and paracasein by biological methods. Three methods were tried, precipitation, complement binding, and anaphylaxis, and of these only complement binding gave positive results under certain conditions. The other two methods entirely failed to distinguish between the two substances. Schryver[2] has suggested that all the substances necessary for clot formation pre-exist in milk and that aggregation is prevented by the absorption of simpler molecules from the system. He formed the conception that a ferment, for which the colloidal substances could act as a substrate, could clear the surface of such substances of adsorbed bodies and thus allow aggregation (clot) formation to take place. He found that milk serum, Witte's peptone, or glycine, inhibited clot formation by rennin, and also that apparently typical milk clots could be formed by the addition of calcium chloride to calcium caseinogenate solutions and warming. These differ from rennin clots, however, in their ability to pro-

duce clottable solutions on dispersion by acidification after solution in alkali. Clots produced by the action of rennin cannot be redispersed, a fact that suggests some alteration in structure. Schryver found that calcium caseinogenate solutions on warming, and sodium caseinogenate solutions after treatment with carbon dioxide in the cold, would produce clots with rennin and suggested that these observations point to the formation of caseinogen by the action of heat in the former, and carbon dioxide in the latter, and that clot formation is produced by the action of rennin on the free caseinogen or metacaseinogen (see p. 7).

Some observers have stated that a change in reaction occurs during the action of rennin but Hewarden [24] found that hydrogen ions were not necessary for the coagulation of milk or solutions of caseinogen containing calcium. The author has found that the curd produced from milk by rennin usually has an acidity equivalent to 8.3 to 8.8 c.cms. of normal acid per litre of milk, an amount which is identical with the acidity of the caseinogen in the solution from which it is produced.

Caseinogen is also clotted by the action of trypsin and other enzymes, but in the case of trypsin there is definite evidence of proteoclastic cleavage with the formation of soluble compounds containing nitrogen and phosphorous.

Heating milk to 70° C. and upwards, retards the velocity of the rennin reaction by partial destruction of the enzyme and precipitation of the calcium salts: refrigeration also prevents the formation of the characteristic curd but this property is regained on heating to 37° C. (Morgenrath).

The optimum reaction temperature for rennin is about 40° C. and at temperatures exceeding this it is gradually weakened and finally destroyed: the destruction by heat follows the law of a monomolecular reaction. The velocity of the rennin reaction follows the usual laws until 40° C. is reached when the observed values become smaller than the calculated values owing to partial weakening of the enzyme by heat. Some of the results obtained by Field on this subject are given in Table IV.

TABLE IV

| Temperature. | T Time in Seconds. | K Observed $\frac{10,000}{T}$. | K Calculated. |
|---|---|---|---|
| 25 | 54 | 185 | 185 |
| 30 | 32 | 312 | 327 |
| 35 | 17 | 588 | 574 |
| 40 | 10.2 | 980 | 980 |
| 44 | 9 | 1111 | 1491 |
| 50 | 14.7 | 680 | 2742 |

The time required for the coagulation of milk by rennin, other conditions being equal, is inversely proportional to the concentration of the enzyme. Acids and salts of the alkaline earths accelerate the reaction, while alkalies, albumoses, and large amounts of neutral salts, retard it: the fat content also influences the velocity of the reaction. The reaction can be inhibited by the addition of normal horse serum and a similar effect is produced by the anti-rennin prepared by Morgenrath [25] by repeated injection of rennin into the blood stream of rabbits. As the inhibitory action of horse serum can be prevented by neutralisation with acid (Raudnitz and Jakoby) it seems probable that both horse serum and anti-serum act by fixation of the calcium ions.

**Lactalbumin.** This constituent of milk has, according to Sebelien, the following composition:

| | Carbon. | Hydrogen. | Nitrogen. | Sulphur. | Oxygen. |
|---|---|---|---|---|---|
| Lactalbumin..... | 52.19 | 7.18 | 15.77 | 1.73 | 23.13 |

These results show that the essential difference in composition between the albumin of milk and the phospho proteid (caseinogen) lies in the absence of phosphorus in the former and its markedly higher content of sulphur.

Lactalbumin follows the general reactions of other albumins in being soluble in neutral saturated solutions of magnesium

sulphate but is precipitated by the addition of small quantities of acetic acid. It is stated that lactalbumin may be obtained in a crystalline form by diluting the saturated magnesium sulphate solution with an equal volume of water and setting aside after the addition of acetic acid until permanently turbid.

Lactalbumin is also precipitated by sodium and ammonium sulphates when added to saturation. Tannin, phosphotungstic acid and other general reagents also precipitate lactalbumin: the salts of the heavy metals are insoluble in water. Lactalbumin is insoluble in alcohol and this reagent may be employed for the precipitation of lactalbumin from aqueous solutions: the precipitate so obtained is easily soluble in water.

Lactalbumin is a white powder possessing neither taste nor odour. It coagulates at 70° C. but the precipitation is never complete. The specific rotatory power, according to Béchamp, is $[\alpha]_D = -67.5$, but Sebelein obtained values varying from $-36.4$ to $-38.0$. Lindet [26] obtained a value of only $-30.0$, so that apparently the preparations of both Béchamp and Sebelein were mixtures of lactalbumin with some other substance, probably caseinogen $[\alpha]_D = -119$, having a much higher rotatory power.

**Lactoglobulin.** Comparatively little is known regarding the globulin constituent of milk. It is precipitated by neutral sulphates such as magnesium sulphate but is quite soluble in sodium chloride solutions even after acidification. It is not clotted by rennin but coagulates under the action of heat alone at a temperature of 72° C. (Hewlett).

Probably not more than 0.1 per cent of lactoglobulin is present in normal milk although considerably more may be found in colostrum.

**Mucoid Proteid.** This substance, according to Storch, contains 14.76 per cent of nitrogen and 2.2 per cent of sulphur. It is a greyish white powder which is slightly soluble in dilute sodium and potassium hydrates though insoluble in ammonium hydrate, acetic, and hydrochloric acids. Mucoid proteid gives the usual proteid reactions with Millon's reagent (red), and

iodine (brown), and the xantho proteic reaction. On hydrolysis with hydrochloric acid it yields a quantity of a substance capable of reducing Fehling's copper solution.

This proteid is probably identical with the $\beta$ casein of Strewe who separated it from $\alpha$ casein (caseinogen) by dissolving out the latter with ammonium hydrate.

**Salts.** In addition to the various acids and bases which form part of the caseinogen complex, the serum of milk contains various salts in solution. The average percentage of ash in milk is about 0.75 per cent but fluctuates considerably. The average composition of the ash of milk, as obtained by ignition is given in Table V.

TABLE V

COMPOSITION OF ASH OF MILK (RICHMOND)

| | Per Cent. |
|---|---|
| Lime | 20.27 |
| Magnesia | 2.80 |
| Potash | 28.71 |
| Soda | 6.67 |
| Phosphoric acid | 29 33 |
| Chlorine | 14.00 |
| Carbon dioxide | 0.97 |
| Sulphuric acid | Trace |
| Ferric oxide | 0.40 |
| | 103.15 |
| Less oxygen = Cl | 3.15 |
| | 100.00 |

Distribution of the phosphoric acid.

| | Grams per 100 c.cms. |
|---|---|
| $P_2O_5$ as caseinogen combined with NaCa | 0.0605 |
| $P_2O_5$ as $Ca_3P_2O_8$ | 0.0625 |
| $P_2O_5$ as $R_3HPO_4$ | 0.0770 |
| $P_2O_5$ as $RH_2PO_4$ | 0.0200 |
| Total | 0.2200 |

The following results of Van Slyke and Bosworth [12] show the composition of milk serum as separated by filtration through porcelain candles.

TABLE VI

COMPOSITION OF MILK AND MILK SERUM

| | Original Milk. | Milk Serum. | Percentage of Milk Constituents in Serum. |
|---|---|---|---|
| Sugar | 5.75 | 5.75 | 100.0 |
| Caseinogen | 3.07 | 0.00 | Nil |
| Albumin | 0.506 | 0.188 | 37.1 |
| Nitrogen in other compounds | 0.049 | 0.049 | 100.0 |
| Citric acid | 0.237 | 0.237 | 100.0 |
| Phosphorus (organic and inorganic) | 0.125 | 0.067 | 53.6 * |
| Phosphorus (organic) | 0.087 | 0.056 | 64.4 |
| Calcium | 0.144 | 0.048 | 33.3 |
| Magnesium | 0.013 | 0.007 | 53.8 |
| Potassium | 0.120 | 0.124 | 100.0 |
| Sodium | 0.055 | 0.057 | 100.0 |
| Chlorine | 0.076 | 0.081 | 100.0 |
| Ash | 0.725 | 0.400 | 55.2 |

* Not obtained on same sample.

Van Slyke and Bosworth suggest that the various combinations of acids and bases in milk are:

| | |
|---|---|
| Proteins combined with calcium | 3.20 |
| Di-calcium phosphate ($CaHPO_4$) | 0.175 |
| Calcium chloride | 0.119 |
| Mono-magnesium phosphate ($MgH_4P_2O_8$) | 0.103 |
| Sodium citrate ($Na_3C_6H_5O_7$) | 0.222 |
| Potassium citrate ($K_3C_6H_5O_7$) | 0.052 |
| Di-potassium phosphate ($K_2HPO_4$) | 0.230 |

Other constituents which have been found in minute traces are fluorine, iodine, silica, acetates, and thiocyanates.

**Lecithin.** $C_{44}H_{90}O_9NP$ also exists in milk in minute quantities.

**Gases.** There is no definite evidence of the existence of gases in milk as drawn from the udder, but, during this process, it absorbs the normal constituents of the air. Two analyses of milk gases by Winter Blyth are given in Table VII.

TABLE VII

COMPOSITION OF GASES IN MILK

| | Fresh Milk. | Milk after Standing Two Hours. |
|---|---|---|
| | Cubic centimeters per 1000 c.cms. of milk | |
| Carbon dioxide | 0.06 | 60.47 |
| Oxygen | 19.13 | 9.30 |
| Nitrogen | 77.60 | 30.21 |

Blyth found that, on standing, the oxygen usually disappeared in about twenty-four hours and that the carbon dioxide content increased until it finally reached over 95 per cent of the total gases, the residue being nitrogen.

**Enzymes.** It has been indubitably proved that fresh milk contains a number of the substances known as enzymes, bodies which are remarkable on account of certain properties which they possess. Small quantities appear to be capable of producing radical chemical changes without themselves undergoing alterations, although their activity is diminished by the transformation products.

Enzymes are specific in character, i.e., only certain specific enzymes are capable of acting upon certain compounds, and this property has led to the adoption of a nomenclature which classifies the enzyme in accordance with the nature of the compound acted upon or the nature of the action produced. For example, the enzyme acting upon amylose is known as amylase, whilst lactase, glucase, and protease, act upon lactose, glucose, and protein, respectively: oxidases and reductases oxidise and reduce, and catalase acts as a catalytic agent.

Enzymes are thermolabile, have optimum temperatures of

reaction, and are injuriously influenced by toxins and various salts. As they have never been isolated in a pure condition, comparatively little is known as to their composition and it is by their properties rather than differences in composition that enzymes are recognised.

Amongst the various enzymes that have been discovered in milk are amylase, galactase, lipase, lactokinase, peroxidase, reductase, and catalase.

**Amylase.** Béchamp [27], in 1883, prepared an amylase from human milk that converted soluble starch into sugar as readily as amylases from other sources. The presence of amylase in cows' milk has been denied by Moro, der Velde, Landtsheer, and Kastle and affirmed by Zaitschick, Koning, Seligman, Jensen, and others. The author has invariably found amylase to be present, although only in minute quantities.

**Galactase.** This protease was first found in milk by Babcock and Russell in 1897 [28]. They found that fresh centrifuge slime showed proteolytic properties even when all bacterial activity was checked by the presence of antiseptics. Wender [29] has shown that the galactase prepared from centrifugal slimes is not a pure enzyme but a mixture of galactase with peroxidases and catalase. The presence of catalase in milk has, however, been confirmed by von Freudenreich, Jensen, Spolverini, and others. The action of galactase on proteids is very similar to that of trypsin, proteoses and peptones being the intermediate, and amino acids the final products.

**Lactokinase,** a kinase similar to enterokinase, and a fibrin ferment have also been found in minute quantities.

**Lipase,** the enzyme capable of hydrolysing glycerides of fatty acids such as monobutyrin, was found in milk by Marfan and Gillet [30]. Cows' milk was found to have a lipolytic activity of 6–8 on Hanriot's scale as against 20–30 for human milk.

**Salolase.** That human and asses' milk have the property of hydrolysing phenyl salicylate (salol) was observed by Nobécourt and Merklen.[31] The existence of this ferment in milk was disputed by Dèsmoulièrs and also by Mule and Willem,

who found that the hydrolysis was really a saponification effected by the presence of alkali and that only alkaline milks showed the presence of salolase. Rullman, in 1910, proved that milk obtained with aseptic precautions did not give the salol splitting reaction. It has been suggested that salolase is of bacterial origin, although this view is unsupported by experimental data.

**Peroxidases.** Although Rullman has found traces of substances in milk capable of effecting oxidation by utilisation of atmospheric oxygen (true oxidases), the peroxidases are much more important. These ferments decompose hydrogen peroxide in accordance with the equation $H_2O_2 = H_2O + O$. The presence of nascent oxygen is ascertained by the addition of some substance which undergoes a colour change on oxidation (a chromogen). Benzidine, guiacol, ortol, amidol, p. phenylenediamine, and phenolphthalin have been employed for this purpose. Kastle and Porch [2] showed that the power of milk to induce the oxidation of phenolphthalin and other leuco bases by hydrogen peroxide is greatly intensified by the addition of certain substances of the phenol type.

**Catalase.** Catalase (Loew) or superoxidase (Raudnitz) like peroxidase has the property of decomposing hydrogen peroxide, but, instead of atomic oxygen being produced and absorbed by some compound present, molecular oxygen is formed and may be collected in the gaseous form.

$$2H_2O_2 = 2H_2O + O_2.$$

Some authors have included catalase with the reductases in accordance with the view that the oxygen liberated is utilised in an oxidation process and that the reaction is essentially one of the reduction of hydrogen peroxide to water. There is, however, as little basis for the inclusion of catalase with the reductases as with the peroxidases, for, although its action is intermediate between the two, it is entirely independent of them and well-defined in character.

**Reductases.** The ferments which cause the abstraction of oxygen from compounds without the production of gaseous oxygen, have been termed reductases. The essential difference between this reaction and that produced by catalases is in the utilisation or transference of the oxygen removed.

Two types of reductase have been recognized and are differentiated by their action on methylene blue. One type, which appears to be of cellular origin and is present in fresh milk, rapidly decolourises methylene blue solutions in the presence of a trace of formaldehyde, whilst the other is capable of effecting the reduction in the absence of formaldehyde and is of bacterial origin.

### Biological

**Immune Bodies.** Although the examination of milk for the presence of immune bodies is but infrequently required in connection with public health work, a general consideration of these bodies and their significance is of interest. Before considering these in detail it will be advisable to review briefly the theory of immunity.

After an attack of disease-producing organisms, animals usually possess, for a varying length of time, an immunity against a further attack, and this immunity is ascribed to the presence of substances known as immune bodies. The researches of Ehrlich and others have established that these immune bodies, or anti-bodies as they are generally described, are produced by external agencies. In addition to living and dead bacteria, other substances such as animal and vegetable proteins, animal cells, and toxins, may act as antigens. Ehrlich's theory of immunity hypothecates the existence, in the molecules constituting both the antigen and body cell, of binding groups or haptophoric receptors which fit "as a key fits the lock" and which anchor the antigen to the body cell. In the case of toxins, other receptors are also assumed to be present, viz., toxophores, which are responsible for the toxic effects produced after the antigen has been anchored to the cell.

The cell molecules may be destroyed as the result of this combination or it may be stimulated by defensive action to the production of receptors; continued excitation results in the production of more receptors than are necessary for the functions of the cell and it is assumed that these receptors are set free in the fluids surrounding the cells, and that they possess a greater affinity for the antigen than the same receptors of the cell molecule. These free receptors constitute the antibodies. Three varieties of antibodies are known.

(1) Uniceptors, such as antitoxins, which are regarded as comparatively simple and which combine directly with the antigen.
(2) Uniceptors, which have an enzyme-producing group in addition to the haptophoric receptor (agglutinins, precipitins).
(2) Amboceptors, which require the presence of a third substance before combination with the antigen can be effected; this third substance is known as complement.

Antigens, and uniceptors produced by them, are specific in their action, and this applies equally to the amboceptor-complement-antigen system of the third order of receptors. For instance, tetanus antitoxin acts on tetanus toxins and on no others, and typhoid serum agglutinates only B. typhosus. This statement, however, is not absolutely true, as antigens produced by allied groups of organisms possess receptors which are common to all, but as the specificity becomes more definite with increased dilution of the antibody, the affinity between the specific receptors must be considered to preponderate. The amboceptors of the third order of antibodies also show relative rather than absolute specificity.

The antibodies generally are distinguishable from complements by their resistance to heat. The uniceptors and amboceptors are thermostabile, i.e., are not destroyed by heating to

6° C. for thirty minutes, whereas complement is destroyed by this treatment; complement is, therefore, thermolabile.

Antibodies, like enzymes, are of unknown chemical constitution and are usually designated by the nature of the action produced; thus, antitoxins neutralise toxins, cytolysins dissolve animal cells, hæmolysins dissolve erythrocytes, bacteriolysins dissolve bacteria, agglutinins agglutinate cells and bacteria, and precipitins produce precipitates from solutions.

Immunity, by which is understood the existence of a certain resistance toward deleterious influences, may be either acquired or natural. The apparent immunity of individuals, races, and species to various diseases under normal conditions is known as natural immunity, and very little is known of the etiological factors involved. Acquired immunity may be accidental, as in the case of the immunity acquired by an attack of a disease, or artificially acquired by the introduction into the system of either antigens or antibodies. When antibodies are employed, the immunity is but of short duration compared with the several years of immunity obtained by the use of antigens. The former process is known as passive immunity and the latter as active immunity.

When *antibodies* are present in the blood, certain quantities are excreted by the milk glands and may be found in the milk. Ehrlich has demonstrated that offspring may, through suckling, obtain a passive immunity from either an actively or passively immunised mother. The antibody content of milk is usually very much weaker than that of the blood from which it is derived. Unieeptors of the second order are also transferable to the milk and may be less than, equal to, or even greater, than the amounts found in the blood. The evidence regarding the transfer of the third order of antibodies is somewhat conflicting. *Amboceptors* and *complement* derived from the blood may appear in the milk, but this is unusual and various experimenters have stated that complement is not present in normal ripe milk except in minute traces. In colostrum and milk derived from udders affected with mastitis, however, both

amboceptor and complement may be present. The application of the complement fixation test for the detection of colostrum is only of scientific interest and mastitis can be much more readily detected by an examination of the sediment of the milk.

*Opsonins*, bodies which prepare bacterins for phagocytosis, the process by which a cell (phagocyte) absorbs bacterins and other particulate matter, have also been demonstrated in milk.

It is possible that *anaphylactins*, which induce the phenomenon known as anaphylaxis or hypersensitiveness, may occur in milk as it has been shown by Otto that the progeny of hypersensitised guinea pigs were anaphylactic to homologous antigens. The transmission, however, may have been either intrauterine or through the milk. Mention might also be made of the beneficial effect upon children suckling from mothers being treated with "606," although whether the results are due to the passage of antibodies or arsenic is still in dispute. Considering the indubitable proof of the passage of various classes of antibodies from the blood stream to milk, it is reasonable to assume that *aggressins*, bodies which inhibit the protective power of the cells, and *toxins* are also transferable. This hypothesis has been experimentally established, but, like the antitoxins, the amounts found in the milk are considerably smaller than in the blood. If it is assumed that the gastro-intestinal tract of infants is penetrated by proteids, the question of the transference of toxins assumes practical importance. Even in individuals showing severe symptoms, by far the greater part of the antigen is anchored to the cell leaving but little in the free or labile condition in the system, and, as only a fraction of this is transferred to the milk, the total amount assimilated by the offspring is probably negligible; à posteriori observations confirm this deduction.

Since milk contains various proteid substances, it is capable of acting as antigen and on injection produces a number of antibodies. The lactoserum obtained by the use of cows' milk contains precipitins, amboceptors, and hæmolysins, which

are specific in their reactions and may be used as qualitative tests for milk. Bauer succeeded in detecting as small a quantity as 1 c.cm. of cows' milk per litre of human milk by the complement fixation method. The various proteids of milk, caseinogen and albumin, etc., also produce specific antibodies which may be recognised by the precipitin method. The specificity of lactoserum, like those of sera in general, is relative rather than absolute, the lactosera of closely related animals being differentiated by the *intensity* of the reactions. The phenomenon of anaphylaxis may also be induced by the injection of milk. Arthus and Besredka state that boiled milk, as well as the raw product, is capable of producing the requisite conditions, though Miessner found that a larger number of injections were necessary before sensitisation was satisfactorily established. Caseinogen and albumin also produce specific anaphylactins which may be used as a basis for differential tests.

**Physical.** The characteristic appearance of milk is produced by the colloidal suspension of caseinogen complex and the emulsion of fat globules. When milk is allowed to remain quiescent, the fat globules, being of smaller density, rise to the surface and form a layer of cream which is distinctly yellowish in tint, the residual milk being bluish white in colour. The opacity is diminished by the addition of alkali, which dissolves the caseinogen, and is increased by any process that reduces the size of the fat globules. Heat alone, at different temperatures, is capable of reducing the diameter of the fat globules, but it may be more effectively accomplished by forcing milk heated to 60° C. through very small orifices under high pressure.

The *specific gravity* of milk bears a definite relation to the total solids it contains (see p. 70), being decreased by the fat content and increased by the solids other than fat. The specific gravity or density varies considerably with variations in season, period of lactation, breed, and character and quantity of food, but 1026.4 to 1037.0 ($^{\text{water}}\frac{15^\circ\text{ C.}}{15^\circ\text{ C.}} = 1000$) may be regarded as the extreme limits. When milk, freshly drawn from the udder,

is allowed to stand for one hour to eliminate air bubbles, it will be found to have a density somewhat lower than that taken subsequently (Recknagel's phenomenon). This peculiarity has been investigated by several observers. Vieth confirmed Recknagel's results and found the average rise to be +1.3° (water = 1000). H. Droop Richmond[33] reports that in 70 per cent of his experiments the rise varied from 0.3° to 1.5°, averaging 0.6°, and that in 30 per cent of the observations no rise in density was indicated; also that the rise was more rapid at low temperatures than at high temperatures. H. D. Richmond, from consideration of experiments made in conjunction with S. O. Richmond on the effect of heat upon the density and specific heat of milk, regards the phenomenon as largely due to the increase in density of the fat on solidification. Changes in the milk sugar, cessation of expansion of the caseinogen, absorption of gases, and enzyme action have also been suggested as causes of this phenomenon but cannot be regarded

TABLE VIII

EFFECT OF TEMPERATURE ON VOLUME

| Temperature in Degrees Fahrenheit. | Volume | Temperature in Degrees Fahrenheit. | Volume. |
|---|---|---|---|
| 31 | 1.00000 | 60 | 1.00229 |
| 35 | 1.00016 | 65 | 1.00298 |
| 40 | 1.00041 | 70 | 1.00372 |
| 45 | 1.00074 | 75 | 1.00451 |
| 50 | 1.00114 | 80 | 1.00549 |
| 55 | 1.00164 | | |

as satisfactory. Various data confirming Richmond's hypothesis were obtained by Toyonaga, and Fleishmann and Weigner.[34] The latter observers found that the change in density was proportional to the amount of butter fat present. Microscopical examinations also showed that the solidified globules were of greater density than the liquid globules at the same temperatures.

Although milk contains considerable quantities of water (85–90 per cent), the maximum density is found at a temperature near to the freezing point and not at 4° C. as in the case of water. The changes in the volume of milk due to temperature alterations are somewhat variable, being dependent upon the composition; the preceding table, due to Richmond, shows the expansion in glass of milk containing 3.8 per cent of fat and having a density of 1032.0.

The *viscosity* of milk, according to Taylor,[35] is not proportional to the percentage of total solids, but is a function of the fat and the solids-not-fat content. He found that the relation is expressed by the formula:

$$\text{percentage solids-not-fat} = \frac{(\text{viscosity} - \text{fat percentage} \times 0.0665)}{0.177},$$

and that the viscosity temperature coefficient was

$$n_t = \frac{n_0}{1} + 0.00723t - 0.000156t^2.$$

Taylor's determinations of the viscosity of milk raised from 20° to 60° C. and subsequently cooled, support the hypothesis of Richmond regarding the explanation of Recknagel's phenomenon. Weigner[36] found that homogenisation of milk slightly increased the viscosity. Two samples having viscosities of 1.941 and 1.862, as determined with an Oswald viscosimeter, were increased by homogenisation to 1.967 and 1.889, respectively. Weigner thought that this was caused by increased adsorption, especially of caseinogen.

The *freezing point* of milk is slightly lower than that of water, being usually −0.54 to −0.57° C. and is especially influenced by the mineral content other than that associated with the caseinogen. As the salts are not subject to wide variation in the milk of healthy cattle, the freezing point is usually fairly constant. This forms the basis of the cryoscopic methods for the detection of milk adulteration. Aitkens[37] shows that a consideration of the osmotic pressure of the blood of animals and that of the milk secreted points to the conclusion that the

freezing point of milk will never fall below that of blood. He found the freezing point of the blood of the cow to be $-0.62°$ C. and that of cows' milk $0.55°$ C.$\pm 0.06°$ C.

In contrast with the relative constancy of the depression of freezing point of cows' milk, *the specific conductivity* shows greater variations, although milk produced under normal conditions does not show very marked differences.

The following results are given by various observers:

TABLE IX

CONDUCTIVITY OF MILK

| | |
|---|---|
| Koeppe (1898) | K at 25° C. = 0.00430–0.00560 |
| Lehnert (1897) | 0.00487–0.00551 |
| Schnorf (1905) | 0.00485 |
| Benaghi (1910) | 0.00494–0.00517 |
| Jackson and Rothera (1914) | 0.00493–0.00641 |
| Jackson and Rothera Herd milk (1914) | = 0.00549–0.00587 |

Jackson and Rothera [38] point out that, owing to the osmotic pressure of milk being controlled by that of the blood, the substances chiefly responsible for this manifestation, viz., the milk sugar and soluble salts, cannot vary independently, but must be inter-related. If the lactose is high the salts must be low, and conversely, if the lactose is low the salts must be high or the osmotic pressure would be lower than normal. Jackson and Rothera found experimentally that the electrical conductivity of milk, which is mainly due to the soluble salts, is inversely proportional to lactose content. This inverse proportionality was especially observable in milk produced under pathogenic conditions, as shown by the following example:

| Quarter. | Conductivity, K | Lactose, Per Cent. | Depression of Freezing-point. Δ | Sol. Ash, Per Cent. | Insol. Ash, Per Cent. |
|---|---|---|---|---|---|
| Left anterior (abnormal). | 0.0114 | 1.50 | 0.580 | 0.615 | 0.440 |
| Right anterior (normal). | 0.00569 | 5.40 | 0.575 | 0.285 | 0.625 |

As the proteins of milk obstruct the carriage of electricity by the moving ions, the conductivity of whey or of serum is greater than that of the milk from which it is prepared. Each 1 per cent of protein reduces the conductivity by 2.75 per cent (Rothera and Jackson). The *surface tension* of milk is lower than that of water, 0.053 as against 0.075 and the *specific heat* of milk containing 3.17 per cent of fat is, according to Fleishmann, 0.9457.

The *refractive index* of milk cannot be determined on account of its opacity, but that of the serum, after removal of the caseinogen and fat, has been determined on a large number of samples by various observers and is now regarded as a valuable aid in the detection of adulteration by the addition of water.

This method is of special value on account of the removal of the constituents of milk that are most variable in amount, viz., fat and caseinogen, leaving a serum containing the lactose, mineral matter, and albumin which are generally the least variable. Various methods, which vary somewhat in the completeness of precipitation of caseinogen attained, have been employed,[39,40] and normal values established for each. The refractive index of fresh milk serum, prepared by filtration through porous plates, varies from ($\mu_D$20° C.) 1.34200 to 1.34275. The specific gravity of milk serum is equally as valuable as the refractive index (see p. 79) but on account of the longer time required for its determination it is not generally used as a routine method. The ash of the serum also affords valuable information for the detection of added water. (Lythgoe,[40] and Burr and Berberich.[41]).

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## CHAPTER II

### THE NORMAL COMPOSITION OF MILK

The average composition of cows' milk as compared with the milk of various other mammals is shown in Table No. X. (Bunge [1]).

Table X

COMPOSITION OF MAMMALS' MILK

| | Fat. | Caseinogen. | Albumin. | Lactose. | Ash. |
|---|---|---|---|---|---|
| Human (1) | 3.1 | ..... | ..... | 5.9 | 0.2 |
| Human (2) | 3.8 | 1.2 | 0.5 | 6.0 | 0.2 |
| Human (3) | 3.3 | ..... | ..... | 6.5 | 0.3 |
| Dog | 12.5 | 5.2 | 1.9 | 3.5 | 1.3 |
| Cat | 3.3 | 3.1 | 6.4 | 4.9 | 0.6 |
| Rabbit | 10.5 | ..... | ..... | 2.0 | 2.6 |
| Guinea pig | 45.8 | ..... | ..... | 1.3 | 0.6 |
| Sow | 6.9 | ..... | ..... | 3.8 | 1.1 |
| Elephant | 19.6 | ..... | ..... | 8.8 | 0.7 |
| Horse | 1.2 | 1.2 | 0.8 | 5.7 | 0.4 |
| Ass | 1.6 | 0.7 | 1.6 | 6.0 | 0.5 |
| Cow | 3.7 | 3.0 | 0.9 | 4.9 | 0.7 |
| Goat | 4.8 | 3.2 | 1.1 | 4.5 | 0.8 |
| Sheep | 6.9 | 5.0 | 1.6 | 4.5 | 0.9 |
| Reindeer | 17.1 | 8.4 | 2.0 | 2.8 | 1.5 |
| Camel | 3.1 | ..... | ..... | 5.6 | 1.8 |
| Llama | 3.2 | 3.0 | 0.9 | 5.6 | 0.8 |
| Porpoise | 54.8 | | 7.6 | | 0.5 |

Apart from the very varying amounts of fat the similarity in the composition of the milk of these various mammals is very remarkable.

Various observers have recorded the results of thousands of analyses of cows' milk and some of the most authentic are given in Table XI.

TABLE XI

COMPOSITION OF COWS' MILK

| Average of | Water. | Fat. | Caseinogen. | Albumin. | Lactose. | Ash. |
|---|---|---|---|---|---|---|
| 280,000 analyses, Aylesbury Dairy Co., London, Richmond | 87.35 | 3.74 | 3.0 | 0.4 | 4.70 | 0 75 |
| 5552 analyses in U. S. A. Van Slyke | 87.10 | 3.90 | 2.5 | 0.7 | 5.10 | 0.70 |
| Cheese factory milk. New York State. May to Nov. Van Slyke | 87 40 | 3 75 | 2 45 | 0.7 | 5 00 | 0.70 |
| 800 analyses by Koenig | 87.27 | 3.64 | 3.02 | 0.53 | 4.88 | 0.71 |

The essential difference between the European and American results lies in the ratio of lactose to proteids and the relative amounts of caseinogen and albumin that make up the total proteids. Numerous analyses by the author of Canadian milk show that the average ratio of lactose to proteid in that country is distinctly higher than those recorded by Richmond and Koenig. The figures of Lythgoe [2] for milk in Massachusetts, confirm this view. At least a portion of the differences between the relative amounts of caseinogen and albumin in the analyses

TABLE XII

MAXIMUM VARIATIONS IN COMPOSITION

| | Fat. | Solids Not-fat. |
|---|---|---|
| | Per cent. | Per cent. |
| Maximum | 14.67 | 13.76 |
| Minimum | 1.04 | 4.90 |

recorded in the above table is probably due to errors in the various methods used for the determination of these constit-

## Table XIII

EFFECT OF BREED ON COMPOSITION OF MILK (Lythgoe)

| | Jersey. | Guernsey. | Grade Jersey. | Grade Guernsey. | Grade Durham. | Grade Ayrshire. | Ayrshire. | Grade Holstein. | Dutch Belt. | Holstein. |
|---|---|---|---|---|---|---|---|---|---|---|
| Total solids | 14.75 | 14.60 | 13.74 | 13.20 | 13.10 | 12.98 | 12.64 | 12.62 | 12.15 | 11.69 |
| Fat | 5.65 | 5.23 | 4.65 | 4.23 | 4.29 | 4.22 | 4.01 | 3.95 | 3.56 | 3.41 |
| Solids-not-fat | 9.10 | 9.37 | 9.09 | 8.85 | 8.81 | 8.76 | 8.63 | 8.67 | 8.59 | 8.28 |
| Lactose | 4.94 | 4.84 | 4.87 | 4.94 | 4.86 | 4.85 | 4.88 | 4.65 | 4.93 | 4.70 |
| Proteids | 3.46 | 3.73 | 3.45 | 3.27 | 3.39 | 3.22 | 2.99 | 3.25 | 2.96 | 2.93 |
| Ash | 0.72 | 0.75 | 0.75 | 0.72 | 0.73 | 0.75 | 0.76 | 0.73 | 0.70 | 0.72 |
| $\frac{\text{Proteid}}{\text{Fat}}$ ratio | 0.61 | 0.71 | 0.74 | 0.75 | 0.79 | 0.76 | 0.75 | 0.82 | 0.83 | 0.86 |
| $\frac{\text{Lactose}}{\text{Proteid}}$ ratio | 1.43 | 1.30 | 1.41 | 1.51 | 1.43 | 1.51 | 1.63 | 1.43 | 1.67 | 1.60 |
| Refraction of serum at 20° C. | | | | | | | | | | |
| Copper serum | 38.1 | 38.2 | 38.0 | 38.0 | 38.0 | 37.9 | 37.7 | 37.6 | 38.3 | 37.2 |
| Acetic serum | 44.2 | 43.9 | 42.9 | 43.7 | 42.7 | 42.5 | 42.8 | 42.5 | 42.8 | 41.6 |
| Sour serum | 42.7 | ..... | 42.2 | 41.7 | 42.1 | 41.9 | 40.5 | 42.0 | ..... | 40.6 |
| Ash of sour milk serum, Grams per litre | 0.786 | ..... | 0.804 | 0.753 | 0.811 | 0.790 | 0.856 | 0.793 | ..... | 0.795 |

uents. Later American analyses have shown that the normal albumin content of 0.7 per cent, as recorded by Van Slyke, is too high and that 0.5 per cent is much nearer the correct value.

**Limits and Variations.** The variation in the composition of milk obtained from herds is not usually very great, but that of individual cows may vary between very wide limits. The following figures show the maximum and minimum that have been recorded, the former by Cook and Hills of milk from a Jersey cow just before going dry, and the latter by Richmond.

The *fat* content of milk is very variable and depends upon a number of factors, the chief of which are breed, food, season, interval between milkings, and stage of lactation.

The *breed* of the cow has a very important bearing upon the quality of the milk produced, some (Jersey and Guernsey) giving milk containing 60 per cent more fat than others (Holstein). Results of analyses of milk from various breeds are recorded in Tables XIII, XIV, and XV.

TABLE XIV

FAT AND SOLIDS NOT-FAT IN MILK FROM VARIOUS BREEDS

(VIETH)

| Breed. | Total Solids. | | | Fat. | | | Solids Not-fat. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average. | Maximum. | Minimum. | Average. | Maximum. | Minimum. | Average. | Maximum. | Minimum. |
| Dairy shorthorn | 12.90 | 18.70 | 10.2 | 4.03 | 10.2 | 1.3 | 8.87 | 10.6 | 7.6 |
| Pedigree " | 12.86 | 16.8 | 10.5 | 4.03 | 7.5 | 1.9 | 8.83 | 9.8 | 7.6 |
| Jersey | 14.89 | 19.9 | 11.0 | 5.66 | 9.8 | 2.0 | 9.23 | 10.4 | 8.1 |
| Kerry | 13.70 | 18.6 | 10.6 | 4.72 | 10.5 | 1.8 | 8.98 | 10.6 | 4.9 |
| Red Polled | 13.22 | 16.2 | 9.7 | 4.34 | 6.6 | 2.5 | 8.88 | 10.2 | 7.1 |
| Sussex | 14.18 | 17.4 | 11.5 | 4.87 | 7.6 | 2.9 | 9.31 | 10.3 | 8.4 |
| Montgomery | 12.61 | 16.1 | 10.2 | 3.59 | 6.5 | 1.4 | 9.02 | 10.0 | 7.9 |
| Welsh | 14.15 | 17.6 | 11.9 | 4.91 | 8.3 | 3.0 | 9.24 | 9.6 | 8.9 |

The figures in Table XV are compiled from results published by the various American Experimental Agricultural Stations.

TABLE XV

| Breed. | Total Solids. | Fat. | Lactose. | Proteid. | Ratio. | |
|---|---|---|---|---|---|---|
| | | | | | $\frac{\text{Lactose}}{\text{Proteid}}$ | $\frac{\text{Proteid}}{\text{Fat}}$ |
| Jersey..... | 14.70 | 5.14 | 5.04 | 3.80 | 1.32 | 0.74 |
| Guernsey..... | 14.49 | 4.98 | 4.98 | 3.84 | 1.30 | 0.77 |
| Ayrshire..... | 12.72 | 3.85 | 5.02 | 3.34 | 1.50 | 0.87 |
| Holstein..... | 12.00 | 3.45 | 4.65 | 3.15 | 1.47 | 0.91 |
| Shorthorn.... | 12.57 | 3.63 | 4.89 | 3.32 | 1.47 | 0.91 |
| Red Poll..... | ..... | 4.03 | | | | |

The influence of breed upon the chemical characteristics of the fat was investigated by Eckles and Shaw [3] and their results are summarised in Table XVI.

TABLE XVI

EFFECT OF BREED ON CHARACTERISTICS OF FAT. (ECKLES AND SHAW)

| Breed. | Relative Size of Fat Globules. | Iodine Number. | Saponification Value. | Reichert-Meissl Value. | Melting-point, Centigrade. |
|---|---|---|---|---|---|
| Jersey..... | 328 | 30.5 | 228.9 | 26.7 | 32.9 |
| Ayrshire..... | 150 | 31.6 | 228.2 | 25.9 | 33 5 |
| Holstein..... | 142 | 34.2 | 229.1 | 25.5 | 32.9 |
| Shorthorn..... | 282 | 34.4 | 227.6 | 26.3 | 33.2 |

It is evident from the results recorded that the breed of cow has a marked effect upon the composition of the milk obtained and that certain constituents are more affected than others. The fat is the most variable constituent, though the total

amount of fat yielded by the various breeds is far less so and is due to the quantity of milk being usually inversely proportional to the fat percentage in the milk. The proportion, however, is not a direct one and it has been proved on many occasions that the breeds giving the low fat percentages yield the largest total weight of fat. For this reason the Dutch, Frisian, and Holstein breeds are very popular for dairy purposes.

Concerning the *effect of food* upon the composition of milk, numerous investigations have been made but the results obtained are apparently somewhat contradictory. This is probably partially due to the conditions under which the experiments were conducted being not strictly comparable. Earlier observers failed to appreciate the fact that a certain weight of fat, proteid, and carbohydrates is necessary for providing body heat and for the repair of waste tissue in the cow, and that this amount is proportional, though not directly so, to the weight of the animal. If the food ration is only slightly in excess of this quantity, the effect of stimulants, such as oil cake, would be to immediately increase both the percentage and total quantity of butter fat secreted; on the other hand, if the ration is sufficient for the body maintenance and milk secretion, additional food would probably not increase either the percentage or the quantity of butter fat, and it is conceivable that they may even be somewhat reduced by this over-feeding process.

Of the more reliable investigations, those of Morgen, Beger, Fingerling, Doll, Hancke, Sieglin, and Zielstorff [4] might be mentioned. They found that food free from fat sufficed for the maintenance of animals in a healthy condition and increased the live weight of the animal, but was totally unsuitable for milk production. The addition of food fat in quantities equivalent to 0.5 to 1.0 gram per kilo of the animal weight favoured the production of milk fat. Later, the first three observers, in a series of experiments extending over six years, obtained results which showed that of all foods, fat alone exerts a specific action on the production of milk fat and that, within certain limits, fat is the most suitable food for butter fat pro-

duction. Malméjac [5] reports the following comparative figures obtained in Algeria from cattle feeding on poor and rich forage.

| | Poor Dry Grass. | Rich Forage. |
|---|---|---|
| Total solids | 11.62–14.25 | 13.76–14.90 |
| Fat | 3.33– 3.50 | 4.05– 4.90 |
| Lactose | 4.53– 5.64 | 4.47– 5.55 |
| Proteid | 3.13– 4.46 | 3.33– 4.54 |
| Ash | 0.60– 0.90 | 0.82– 0.93 |

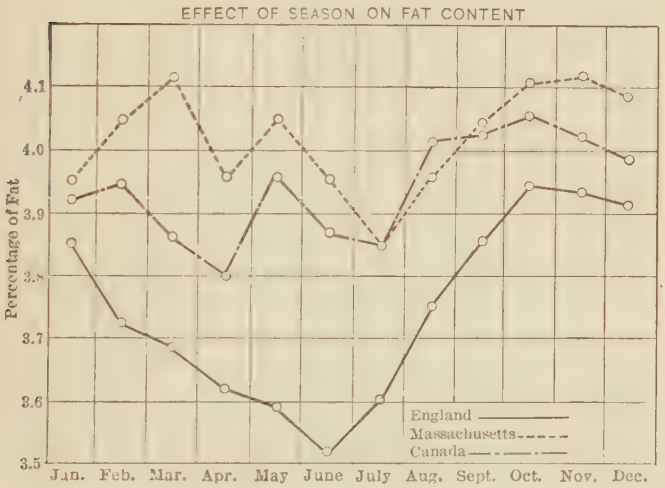
Brewery and distillery waste grains in the wet condition have often been fed to cows on account of the low price of this material, but this procedure ultimately proves to be false economy, as both the relative and absolute amount of milk fat produced is reduced. During the last decade there has been a decided tendency towards scientific feeding of dairy animals with a well balanced ration which is just sufficient for the maintenance of body weight and also for the production of a definite quantity of milk containing a specified amount of butter fat. In this ration, digestibility, palatability, and proportion of roughage to concentrates, are considered and calculated. An example of this rational feeding is seen in the herds of the Minnesota Experimental Station, as compared with the other herds of the state. The common cows, i. e., cows with no dairy heredity, of the Experimental Station yielded 5000 lbs. of milk equal to 222 lbs. of butter per head as against 4000 lbs. of milk equal to 175 lbs. of butter per head for the whole State of Minnesota.

Stable or byre conditions, fatigue, and temperature, also have slight effects upon the fat content of the milk produced.

The *seasonal variation* in the amount of butter fat in milk, according to Droop Richmond's figures, is well marked and always occurs; he finds that the fat content usually decreases during the spring and summer months, reaching a minimum about midsummer, and then gradually rises to a maximum

during the winter. The fat content of the milk of Massachusetts and Ontario shows several modes during the year, although the average values for the summer months are less than those for the winter months. Diagram No. I shows the monthly variations in England for 1897–1913, as compiled by Richmond, in Massachusetts as reported by Lythgoe of State Board of Health, and in Ontario as calculated from the Ottawa analyses of the author. Lythgoe has suggested that

DIAGRAM NO. I




the irregularities in the curve for the Massachusetts supply, as compared with Richmond's results, are due to the larger number of samples examined by the latter. Lythgoe's results, however, are calculated from approximately 13,000 samples examined during three years, and the similarity of the curve to that plotted from the author's analyses of over 9000 samples suggests that the number of modes in the curves is not fortuitous, but is due to seasonal variations together with variations caused by changes of food peculiar to local climatic conditions.

Richmond also found that there were slight daily variations

in the quality of milk, the fat content of Monday's milk being usually slightly lower than that of the other days, but this is apparently due to the usual intervals between milkings being slightly disturbed during the week-end.

The *intervals elapsing between milkings* have been shown by various observers to have an influence on the percentage of fat, though relatively little on the absolute amount. Fleischmann [6] found morning milk slightly richer than evening milk and decided that the fat content varied with the intervals between milking. Richmond [7] as the result of over 100,000 analyses made during sixteen years, gives the figures for the fat content of morning and evening milk as 3.56 and 3.93 per cent, respectively; the intervals being 10.8 and 13.2 hours. His results also show that the difference is more marked during the summer months. Eckles and Shaw [8] found that with equal intervals between milkings, the morning milk was slightly higher in fat content than the evening milk. The Reichert-Meissl and Koettstoffer numbers of the butter fat were usually lower, and the iodine number usually higher in the evening milk, while no appreciable constant variation could be detected in the physical characteristics. With animals milked more than twice daily, the variations in the fat content of the milk were larger and the highest value was usually found in the milk drawn near the middle of the day. The explanation of this is probably connected with the interval between feeding and milking.

The influence of the *stage of lactation* upon the fat content of milk has been the subject of much experimental work, and although some of the data is slightly contradictory, it has been generally established that the percentage of fat usually decreases during the first three months of lactation, then remains fairly constant for four to five months, and, finally, rises rapidly to a maximum. This process is well illustrated by the results of Eckles and Shaw [9] which are given in Table XVII.

The chemical and physical characteristics of the butter fat obtained in these experiments are recorded in Table XVIII.

## Table XVII

AVERAGE PERCENTAGE OF FAT BY FOUR-WEEK PERIODS

| | Jersey. | Shorthorn. | Ayrshire. | Holstein. |
|---|---|---|---|---|
| First period | 5.20 | 4.08 | 3.94 | 3.14 |
| Second | 4.91 | 3.88 | 3.68 | 2.87 |
| Third | 5.02 | 3.71 | 3.60 | 2.78 |
| Fourth | 4.79 | 3.54 | 3.59 | 3.11 |
| Fifth | 4.88 | 3.56 | 3.70 | 3.11 |
| Sixth | 4.98 | 3.58 | 3.52 | 2.98 |
| Seventh | 4.93 | 3.69 | 3.63 | 3.03 |
| Eighth | 4.83 | 3.73 | 3.74 | 3.09 |
| Ninth | 4.84 | 4.19 | 3.71 | 3.05 |
| Tenth | 4.88 | 4.19 | 4.05 | 3.31 |
| Eleventh | 5.23 | 4.11 | 4.92 | 3.39 |
| Twelfth | 5.68 | ..... | 3.96 | 3.70 |
| Thirteenth | 5.48 | ..... | 4.18 | 4.48 |
| Fourteenth | 6.47 | ..... | ..... | 3.68 |

## Table XVIII

RELATION OF LACTATION PERIOD TO CHEMICAL AND PHYSICAL CONSTANTS OF FAT

Average Determinations by Four-week Periods

| Period. | Percentage of Fat. | Relative Size of Globules. | Melting point, Centigrade. | Iodine Number. | Reichert-Meissl Number. | Saponification Number. |
|---|---|---|---|---|---|---|
| 1 | 4.00 | 357 | 31.7 | 33.3 | 29.1 | 223.7 |
| 2 | 3.85 | 307 | 32.9 | 31.6 | 27.5 | 230.4 |
| 3 | 3.79 | 249 | 32.8 | 32.2 | 27.1 | 231.0 |
| 4 | 3.77 | 256 | 33.1 | 30.8 | 26.4 | 229.6 |
| 5 | 3.82 | 200 | 33.3 | 31.4 | 26.6 | 229.2 |
| 6 | 3.79 | 204 | 33.2 | 31.7 | 26.4 | 228.9 |
| 7 | 3.83 | 201 | 33.3 | 32.9 | 25.5 | 225.7 |
| 8 | 3.85 | 192 | 33.4 | 33.3 | 22.2 | 226.7 |
| 9 | 3.97 | 180 | 33.5 | 34.6 | 24.2 | 225.6 |
| 10 | 4.11 | 152 | 33.9 | 35.4 | 22.5 | 223.4 |
| 11 | 4.22 | 162 | 34.7 | 35.5 | 22.2 | 223.8 |
| 12 | 4.54 | 166 | 33.8 | 35.2 | 20.3 | 220.6 |
| 13 | 4.66 | 110 | 36.5 | 39.2 | 17.2 | 216.6 |

It was found that the size of the globules at the commencement of lactation was about twice the average size for the whole period; the size sharply diminished during the first six weeks and then, after remaining fairly constant for some months, rapidly declined. The iodine value varied directly with the fat content, and the saponification value, after a preliminary rise, declined slowly, but gradually, with the constantly decreasing proportion of volatile fatty acids. The melting point remained comparatively steady until the last periods when a perceptible rise occurred; the refractive index showed no appreciable variations.

**Non-fatty Solids.** The non-fatty solids of milk are subject to variations from causes similar to those which determine the variation in the fat content. The influence of *breed* is shown in Tables XIII, XIV, and XV, and that of season in Table XIX (Richmond [10]).

TABLE XIX

INFLUENCE OF SEASON ON SOLIDS-NOT-FAT

| Month. | Fat. | Solids-not-fat. | Lactose. | Proteid. | Ash. |
|---|---|---|---|---|---|
| January | 3.80 | 8.95 | 4.62 | 3.57 | 0.76 |
| February | 3.70 | 8.97 | 4.70 | 3.52 | 0.75 |
| March | 3.62 | 9.91 | 4.72 | 3.45 | 0.74 |
| April | 3.62 | 8.83 | 4.66 | 3.42 | 0.75 |
| May | 3.47 | 8.85 | 4.64 | 3.47 | 0.74 |
| June | 3.44 | 8.82 | 4.68 | 4.42 | 0.74 |
| July | 3.59 | 8.67 | 4.69 | 3.23 | 0.75 |
| August | 3.72 | 8.55 | 4.59 | 3.25 | 0.71 |
| September | 3.88 | 8.63 | 4.63 | 3.25 | 0.74 |
| October | 3.91 | 8.76 | 4.63 | 3.38 | 0.75 |
| November | 3.94 | 8.81 | 4.63 | 3.42 | 0.76 |
| December | 3.80 | 8.81 | 4.56 | 3.50 | 0.75 |

These results show that the solids-not-fat decline sympathetically with the fat during the spring and summer months,

and increase during the autumn and winter seasons. The separation of the constituents forming the non-fatty portion of solids makes it apparent that the decrease in the summer months is due chiefly to the smaller proteid content, the lactose and ash remaining fairly constant. The author's results for Ottawa milks also show a tendency towards a decline in the non-fatty solids during the summer months, though the variations are more irregular than in the series of Richmond given above. In these results the proteid was also the greatest variant and usually accompanied the variations in the fat content.

Richmond [11] found no difference between the non-fatty solids of evening and morning milk on calculating the average results for a number of years. Eckles and Shaw [8] also found no appreciable difference in the total amount of non-fatty solids in morning and evening milk, but their results show that this is due to an increase of proteid in the morning milk with an equivalent reduction in the lactose content.

The effect of the *stage of lactation* upon the solids-not-fat has also been reported upon by Eckles and Shaw.[9] The lactose remained comparatively constant (vide Table XIX) during the greater portion of the period with a slight decline during the last two to three months. The ash was constant and the proteid decreased and increased sympathetically with the fat though not in direct proportion to it.

This sympathetic relation between the amount of fat and proteid in milk has led to the introduction of several formulæ for the calculation of the proteid content from that of the fat. Timpe suggested the formula $P=2+0.35F$ in which $P$ and $F$ represent the percentages of proteid and fat, and gave many analyses in support of it, but Richmond has pointed out that when the series is extended, the agreement practically disappears. Van Slyke's formula [12] $P=0.4(F-3.0)+2.8$, is to be preferred to that of Timpe but cannot be considered as entirely satisfactory. These formulæ are calculated from the averages of many analyses and represent the average relation between fat and proteid in normal milk. Whilst this is of considerable

TABLE XX

INFLUENCE OF STAGE OF LACTATION ON COMPOSITION BY FOUR-WEEK PERIODS

| Period No. | Fat. | Lactose. | Proteid. | Total Solids. |
|---|---|---|---|---|
| 1 | 4.00 | 4.87 | 2.68 | 12.74 |
| 2 | 3.85 | 4.84 | 2.36 | 12.26 |
| 3 | 3.79 | 4.94 | 2.49 | 12.29 |
| 4 | 3.77 | 4.82 | 2.49 | 12.24 |
| 5 | 3.82 | 4.80 | 2.62 | 12.35 |
| 6 | 3.79 | 4.75 | 2.68 | 12.50 |
| 7 | 3.83 | 4.88 | 2.68 | 12.61 |
| 8 | 3.85 | 4.83 | 2.74 | 12.70 |
| 9 | 3.97 | 4.62 | 2.87 | 12.78 |
| 10 | 4.11 | 4.55 | 3.06 | 13.16 |
| 11 | 4.22 | 4.74 | 3.19 | 13.46 |
| 12 | 4.54 | 4.91 | 3.38 | 14.04 |
| 13 | 4.66 | 4.70 | 3.64 | 14.23 |
| 14 | 5.08 | 5.01 | 3.70 | 15.29 |

scientific interest, it is of comparatively little value to the milk examiner who is required to give an expression of opinion upon analytical results with reference to sophistication. Such samples may be derived from many sources and their composition influenced by many factors concerning which he has little or no information; the examiner is, therefore, more vitally interested in the natural variations from the average than in the average itself.

Reference has previously been made to various factors which produce variation in the composition of milk but it is advisable to discuss in more detail their effect upon the relative proportions of the various constituents. The effect of *breed* upon the $\frac{\text{proteid}}{\text{fat}}$ and $\frac{\text{lactose}}{\text{proteid}}$ ratios together with the percentage of fat in the total solids is shown in tables XXI, XXII, and XXIII.

Although some of these results are somewhat discordant, the general tendency is usually in the same direction. When

TABLE XXI

$\frac{\text{PROTEID}}{\text{FAT}}$ RATIO

| Breed. | Van Slyke. | Lythgoe. | Eckles and Shaw. | New Jersey Expt. Stat. |
|---|---|---|---|---|
| Holstein. Frisian | 0.87 | 0.86 | 0.95 | 0.93 |
| Dutch belt | ..... | 0.83 | | |
| Ayrshire | 0.82 | 0.75 | 0.88 | 0.93 |
| American Holderness | 0.83 | | | |
| Shorthorn | 0.80 | ..... | 0.96 | 0.89 |
| Devon | 0.80 | | | |
| Guernsey | 0.66 | 0.71 | ..... | 0.78 |
| Jersey | 0.64 | 0.61 | 0.74 | 0.83 |

TABLE XXII

$\frac{\text{LACTOSE}}{\text{PROTEID}}$ RATIO

| Breed. | Lythgoe. | Eckles and Shaw. | New Jersey Expt. Stat. |
|---|---|---|---|
| Holstein. Frisian | 1.60 | 1.54 | 1.43 |
| Dutch belt | 1.67 | | |
| Ayrshire | 1.63 | 1.51 | 1.39 |
| Shorthorn | ..... | 1.39 | 1.47 |
| Guernsey | 1.30 | ..... | 1.22 |
| Jersey | 1.43 | 1.21 | 1.22 |

the figures are considered in relation to the fat content yielded by each breed, it will be found that, with an increasing percentage of fat, the $\frac{\text{proteid}}{\text{fat}}$ and $\frac{\text{lactose}}{\text{proteid}}$ ratios decrease and that the percentage of fat in the total solids increases.

Seasonal variations are also shown by the various ratios as will be seen from Table XXIV, the figures in which are calculated from those in Table XIX.

## Table XXIII

### PERCENTAGE OF FAT IN TOTAL SOLIDS

| Breed. | Vieth. | Lythgoe. | Eckles and Shaw. | American Expt. Stat. |
|---|---|---|---|---|
| Jersey | 38.0 | 38.3 | 39.2 | 34.9 |
| Guernsey | ..... | 35.9 | ..... | 34.9 |
| Welsh | 34.7 | | | |
| Sussex | 34.4 | | | |
| Kerry | 34.5 | | | |
| Dairy shorthorn | 31.3 | | | |
| Pedigree shorthorn | 31.4 | | | |
| Shorthorn | ..... | ..... | 29.4 | 29.3 |
| Red polled | 32.8 | | | |
| Ayrshire | ..... | 31.8 | 29.6 | 28.7 |
| Dutch belt | ..... | 30.9 | | |
| Montgomery | 28.5 | | | |
| Holstein | ..... | 29.2 | 27.2 | 28.1 |

## Table XXIV

### SEASONAL VARIATIONS IN PROPORTIONS OF CONSTITUENTS

| Month. | $\frac{\text{Proteid}}{\text{Fat}}$ | $\frac{\text{Lactose}}{\text{Proteid}}$ | Percentage of Fat in Total Solids. |
|---|---|---|---|
| January | 0.94 | 1.29 | 29.8 |
| February | 0.95 | 1.33 | 29.2 |
| March | 0.95 | 1.37 | 28.8 |
| April | 0.95 | 1.33 | 29.1 |
| May | 1.00 | 1.31 | 28.4 |
| June | 0.99 | 1.34 | 28.1 |
| July | 0.90 | 1.42 | 29.3 |
| August | 0.87 | 1.41 | 30.3 |
| September | 0.84 | 1.42 | 31.0 |
| October | 0.86 | 1.37 | 30.9 |
| November | 0.87 | 1.35 | 30.9 |
| December | 0.92 | 1.30 | 30.2 |

The influence of the *stage of lactation* upon the various ratios is shown in Table XXV, which is based on the results recorded in Table XVII.

TABLE XXV

INFLUENCE OF STAGE OF LACTATION ON PROPORTIONS OF CONSTITUENTS

By Four-week Periods

| Period No. | $\frac{\text{Proteid}}{\text{Fat}}$ | $\frac{\text{Lactose}}{\text{Proteid}}$ | Percentage of Fat in Total Solids. |
|---|---|---|---|
| 1 | 0.67 | 1.47 | 31.4 |
| 2 | 0.61 | 1.58 | 31.4 |
| 3 | 0.66 | 1.62 | 30.8 |
| 4 | 0.66 | 1.55 | 30.8 |
| 5 | 0.69 | 1.50 | 30.9 |
| 6 | 0.71 | 1.46 | 30.3 |
| 7 | 0.70 | 1.50 | 30.4 |
| 8 | 0.71 | 1.46 | 30.3 |
| 9 | 0.72 | 1.32 | 31.1 |
| 10 | 0.74 | 1.22 | 31.2 |
| 11 | 0.76 | 1.23 | 31.4 |
| 12 | 0.74 | 1.22 | 32.3 |
| 13 | 0.78 | 1.13 | 32.8 |
| 14 | 0.73 | 1.23 | 33.2 |

The above results show that the general tendency during the period of lactation is for the proteid to increase with the fat, though at a slightly higher rate. This increased $\frac{\text{proteid}}{\text{fat}}$ ratio with increase of fat percentage, however, is not capable of general application as the results show that the reverse is the case when the increase in fat is due to the breed of the cow. The lactose content being comparatively constant, its ratio to that of the proteid is reduced with increase of percentage of fat owing to the increased proteid content. The percentage of fat in the total solids increases with the fat as the extra incre-

ment of proteid is more than counterbalanced by the constancy in the lactose and mineral matter.

The percentage of *ash* in milk is comparatively constant but small variations are observable and depend upon variations in the proteid content, as a portion of the ash is combined with the caseinogen to form the caseinogen complex. Richmond has deduced the formula $A=0.36+0.11P$, in which $A$ and $P$ represent the percentages of ash and proteid, for the calculation of the ash content.

It is upon the above basic relations between the amounts of the various constituents in milk that the formulæ of Van Slyke, previously referred to, and that of Olsen [13], $P=T.\ S.-\frac{T.\ S.}{1.34}$ are based. Lythgoe has suggested that lactose may be calculated from the following formulæ.

$$L=T.\ S.-\left[F+0.7+\left(T.\ S.-\frac{T.\ S.}{1.34}\right)\right],$$

from Olsen's formula and

$$L=T.\ S.-[F+0.7+\{0.4(F-3)\}+2.8],$$

from Van Slyke's formula. The ash in these formulæ is assumed to be 0.70 per cent, but it would be preferable to substitute Richmond's formula of $A=0.36+0.11P$ for the assumed value.

All the foregoing refers only to whole milk, that is, the mixed milk obtained by continuous milking until the udders are dry. The variations due to partial milking are very striking and

TABLE XXVI

(BOUSSINGAULT)

| Portion. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Total solids | 10.47 | 10.75 | 10.85 | 11.23 | 11.63 | 12.67 |
| Fat | 1.70 | 1.76 | 2.10 | 2.54 | 3.14 | 4.08 |
| Solids-not-fat | 8.77 | 8.99 | 8.75 | 8.69 | 8.45 | 8.59 |

may be much greater than those caused by the various factors previously discussed. Analyses showing the composition of milk obtained at various stages are given in Tables XXVI, XXVII and XXVIII.

TABLE XXVII

AYRSHIRES (AUTHOR)

| | Fore Milk. | Middle Milk. | Strippings. |
|---|---|---|---|
| Fat | 1.40 | 5.90 | 9.80 |
| Lactose | 4.95 | 4.94 | 4.87 |
| Proteid | 3.17 | 2.98 | 2.78 |
| Ash | 0.80 | 0.74 | 0.71 |
| Ratios $\frac{\text{Lactose}}{\text{Proteid}}$ | 1.57 | 1.66 | 1.75 |
| $\frac{\text{Proteid}}{\text{Fat}}$ | 2.26 | 0.51 | 0.28 |

TABLE XXVIII

AVERAGE OF JERSEYS, SHORTHORNS, AND HOLSTEINS

(ECKLES AND SHAW)

| | Fat. | Lactose. | Proteid. | Ash. | Total Solids. | Relative Size of Fat Globules. |
|---|---|---|---|---|---|---|
| Fore milk... | 1.87 | 5.30 | 3.58 | 0.75 | 10.47 | 139 |
| Strippings.. | 6.28 | 5.33 | 3.38 | 0.70 | 14.86 | 215 |

The physical and chemical characteristics of the butter fat as determined by Eckles and Shaw were as follows:

TABLE XXIX

| | Reichert-Meissl Number. | Iodine Number. | Saponification Number. | Melting point. | Yellow Colour. |
|---|---|---|---|---|---|
| Fore milk... | 27.2 | 34.1 | 230.1 | 33.9 | 39 |
| Strippings.. | 26.3 | 33.8 | 228.3 | 33.9 | 39 |

All these results show that the chief variation in the composition of milk at various stages of milking is due to fat, and that the relative proportions of the plasma constituents remain comparatively constant. The proteid is the most variable component of the plasma and this fact is reflected in the increasing $\frac{\text{lactose}}{\text{proteid}}$ ratio and the decreasing ash percentage. The $\frac{\text{proteid}}{\text{fat}}$ ratio is entirely different to those previously stated and shows the entire lack of organic relation between these two constituents. This points to the variation in the fat content being due to mechanical causes and not to changes in metabolism. This is also the view of Kirchner,[14] who considered that the fat globules are mechanically retained in the fine ducts of the udder and escape in the strippings. Eckles and Shaw point out, in support of this, that the larger the production of milk the greater the increase in fat as the milking proceeds; which is explained by the hypothesis that, in the heavier milking cows, the udder is more congested and the openings of the ducts reduced by compression. The relative size of the fat globules at various stages of milking also supports this view.

**Colostrum.** The name " colostrum " is applied to the udder secretion before, and immediately after, parturition. A yellow viscous secretion, not unlike that produced by pathological conditions, is often formed, but this is replaced several days before parturition by the colostrum proper. Colostrum is a yellowish, sometimes reddish (due to the presence of blood), slimy liquid with an acid reaction and which shows a tendency to separate. Compared with ripe milk the quantity of proteids in colostrum is very high and is due more to increases in albumin nuclein, and globulin than to an excess of caseinogen. This points to glandular inflammation as a result of physiological irritation. Cholesterol, lecethin, creatinine, tyrosine, and urea are also present. Dextrose is present in addition to lactose, which is slightly diminished in quantity, and the ash is higher

than in normal milk. The microscopical appearance of colostrum is characterised by the presence of glandular epithelium

TABLE XXX

COMPOSITION OF COLOSTRUM (SOTHURST)

| Milking Number | Total Solids | Ash. | Fat. | Sugar. | Total Proteid. | Caseinogen. | Globulin. | Albumin. |
|---|---|---|---|---|---|---|---|---|
| 1 | 22.87 | 1.03 | 2.30 | 2.74 | 12.23 | 4.86 | 5.32 | 1.45 |
| 2 | 16.23 | 0.87 | 2.49 | 2.85 | 6.97 | 3.35 | 2.04 | 1.01 |
| 3 | 15.16 | 0.86 | 3.41 | 3.37 | 5.82 | 3.09 | 1.45 | 0.75 |
| 4 | 15.19 | 0.82 | 4.74 | 3.62 | 4.69 | 2.70 | 0.66 | 0.78 |
| 5 | 15.74 | 0.82 | 5.10 | 3.63 | 4.01 | 2.61 | 0.55 | 0.52 |
| 6 | 15.75 | 0.82 | 4.55 | 3.86 | 4.04 | 2.56 | 0.48 | 0.49 |
| 7 | 15.72 | 0.80 | 5.49 | 3.92 | 3.46 | 2.21 | 0.31 | 0.62 |
| 8 | 15.62 | 0.80 | 5.47 | 4.57 | 3.36 | 2.17 | 0.27 | 0.61 |
| 9 | 15.47 | 0.82 | 5.62 | 4.22 | 3.35 | 2.15 | 0.25 | 0.59 |
| 11 | 15.97 | 0.84 | 5.04 | 3.82 | 3.52 | 2.52 | 0.22 | 0.59 |
| 14 | 16.55 | 0.84 | 5.15 | 5.00 | 3.21 | 2.20 | 0.20 | 0.56 |
| 16 | 16.28 | 0.83 | 4.90 | 5.01 | 3.32 | 2.34 | 0.19 | 0.55 |
| 17 | 16.06 | 0.81 | 4.79 | 4.87 | 3.24 | 2.25 | 0.19 | 0.56 |

TABLE XXXI

COMPOSITION OF COLOSTRUM (ENGLING)

| | Immediately After Calving. | After 10 Hours. | After 24 Hours. | After 48 Hours. | After 72 Hours. |
|---|---|---|---|---|---|
| Specific gravity | 1.068 | 1.046 | 1.043 | 1.042 | 0.035 |
| Total solids | 26.83 | 21.23 | 19.37 | 14.19 | 13.36 |
| Caseinogen | 2.65 | 4.28 | 4.50 | 3.25 | 3.33 |
| Albumin and globulin | 16.56 | 9.32 | 6.25 | 2.31 | 1.03 |
| Fat | 3.53 | 4.66 | 4.75 | 4.21 | 4.80 |
| Lactose | 3.00 | 1.42 | 2.85 | 3.46 | 4.10 |
| Ash | 1.18 | 1.55 | 1.02 | 0.96 | 0.82 |

in the form of foam cells and signet ring-shaped cells with so-called moons and caps, and in albuminophores. Numerous

leucocytes are present and also, during the first few days, large numbers of erythrocytes.

The composition of colostrum is shown in Tables XXX and XXXI.

According to Jensen the amylase and catalase content is increased during the colostral period but reductase is absent.

## Abnormal and Adulterated Milk

**Influence of Disease.** Although the chemical examination of milk produced under pathological conditions is of but little practical importance owing to the infrequency with which such conditions exist and the improbability of this milk being sold unmixed with normal milk, it is, nevertheless, of interest to consider the general changes that occur. Acute diseases associated with great pain and fever are usually characterised by a rapid diminution in the quantity of milk secreted. In general and specific infections the fat may be either increased or decreased with similar fluctuations in the ash and lactose contents. According to Schnorf, most of the internal infections, even when the udder is not involved, produce a diminution in the lactose and proteid content as a result of increased metabolism. Catalase, especially in peritonitis and tuberculosis, may be considerably increased and changes in taste and coagulability may result from general infections.

Although it is well known that the composition of milk changes with alterations in the function and condition of the secreting organs, comparatively little is known regarding the influence of diseases of the udder upon the various constituents of the milk. Many analyses have been made and various observers have obtained what are apparently discordant results, but this may be attributed to factors such as intensity and duration of the disease being different.

In acute forms of mastitis, caused by organisms of the colon group, or streptococci, or in mixed infections, the milk may have a bloody discolouration, later becoming more like colos-

trum in appearance and finally changing to a thick yellowish secretion containing many dark flakes in a clear serum.

In chronic infections the changes are gradual and the appearance and composition of the milk may be almost normal for a time; sooner or later, however, the cell content is increased with a consequent increase in the albumin, and erythrocytes cause a discolouration of the sediment on standing.

In udder infections the fat usually decreases but may fluctuate rapidly within rather wide limits; the lactose and caseinogen usually decrease slightly, but the decrease in the latter constituent is more than counterbalanced by a marked increase in the albumin, resulting in an abnormally high proteid content. These changes result in very abnormal $\frac{\text{proteid}}{\text{fat}}$ and $\frac{\text{lactose}}{\text{proteid}}$ ratios as is shown in Table XXXII. On account of the bacterial origin of these infections, the enzymes in the milk are very much increased.

TABLE XXXII

EFFECT OF DISEASE ON COMPOSITION OF MILK

(SCHAFFER AND BENDZYNSKI)

| Character of Disease. | Total Solids. | Fat. | Lactose. | Proteid. | Ash. | $\frac{\text{Proteid}}{\text{Fat}}$. | $\frac{\text{Lactose}}{\text{Proteid}}$. |
|---|---|---|---|---|---|---|---|
| Non infectious garget... | 7.17 | 0.82 | 0.53 | 4.01 | 0.79 | 4.89 | 0.13 |
| Yellow garget..... | 10.66 | 1.99 | 1.84 | 6.00 | 0.83 | 3.01 | 0.31 |
| Parenchymatous mastitis | 9.74 | 2.16 | 1.01 | 4.21 | 0.99 | 1.99 | 0.24 |

Another cause of abnormal composition of milk is the cessation of the lactation period. This has already been discussed on page 49 where it was shown that during the last stages of lactation, the $\frac{\text{lactose}}{\text{proteid}}$ ratio decreased considerably owing to the increased proteid percentage.

**Milk Adulteration.** Artificial abnormalities in the composition of milk produced by the addition of extraneous sub-

stances or by the abstraction of the natural constituents, generally by human agency, is usually conveyed by the term milk adulteration, and this, strictly speaking, has no reference to any standard that may be adopted.

For the detection of adulteration, a complete determination of the various constituents of the sample should be made and the amounts of fat, lactose, proteid, and ash so found compared with the percentages as calculated from the formulæ of Van Slyke, Olsen, and Richmond. The $\frac{\text{proteid}}{\text{fat}}$ and $\frac{\text{lactose}}{\text{proteid}}$ ratios should also be calculated. The addition of water does not give proteid values which are materially different from those calculated by the Olsen formula but are invariably less than those calculated by the Van Slyke formula, the difference being proportional to the amount of water added. The *PVS* (proteid calculated by the Van Slyke method) in this case, is greater than the *P. O.* (proteid calculated by the Olsen method). The addition of water leaves the $\frac{\text{proteid}}{\text{fat}}$ and $\frac{\text{lactose}}{\text{proteid}}$ ratios unchanged.

The amount of proteid found by direct estimation in the case of abstraction of fat would be greater than either of the calculated values, and in this case *P.O.* would be greater than *PVS*. This is due to the Van Slyke formula being based on the constituent which has been abstracted. The $\frac{\text{lactose}}{\text{proteid}}$ ratio would be normal and the $\frac{\text{proteid}}{\text{fat}}$ ratio abnormally high. In both of these instances the $\frac{\text{lactose}}{\text{proteid}}$ ratio is unaltered and this is valuable in distinguishing between naturally abnormal milks and those rendered abnormal by external agencies. High $\frac{\text{lactose}}{\text{proteid}}$ ratios are extremely rare but low ones may be produced by the various causes previously mentioned.

The refractive power of the serum should also be considered

in connection with samples suspected of being adulterated. The index of refraction is reduced by the addition of water but is unaltered by fat abstraction. The following are the minimum figures for genuine milks when prepared by the usual methods.

TABLE XXXIII

REFRACTOMETER VALUES FOR MILK SERUM

| Method of Preparation of Serum. | Reading on Zeiss Immersion Refractometer. |
|---|---|
| Copper sulphate | 36 |
| Acetic acid | 40 |
| Natural souring | 38 |

Although the above methods are capable of detecting the abstraction of small quantities of fat, their possibilities regarding the indication of added water are more limited, and it is doubtful if they could be relied upon to detect additions smaller than would be necessary to reduce the total solids or solids-not-fat below the requirements of any reasonably high standard. Even though these methods are reliable for the detection of the abstraction of small amounts of fat, the advisability of using them as a basis for the certification of adulteration, when the fat exceeds the standard, is extremely doubtful owing to the difficulty of securing a conviction. Those whose duties embrace the analysis of public milk supplies meet many of these examples and have, unfortunately, no option but to report them as genuine, although they are undoubtedly sophisticated. This is one of the inherent disadvantages of minimum standards.

The addition of cane sugar or dextrin to watered milk for the purpose of increasing the non-fatty solids is indicated by a low proteid value, an abnormally high $\frac{\text{lactose}}{\text{proteid}}$ ratio, and a deficiency of ash. Methods for the detection and estimation of cane sugar are given on page 88. Glycerine and starch

have also been employed as counterfeits for non-fatty solids reduced by the addition of water.

### Calculation of Adulteration

**Added Water.** The probable amount of water added to milk may be calculated from the formula

$$\text{Added water} = 100 - \frac{SNF}{snf} \times 100$$ in which $SNF$ represents the amount of solids-not-fat found, and $snf$ the average amount of solids-not-fat found in genuine milks during the same season. If such records are not available a value of 8.8 may be assumed. Where minimum standards are in force the value in the standard is substituted in the above formula, whether it be for solids-not-fat or total solids. Thus

$$\text{Added water} = 100 - \frac{SNF \text{ found}}{\text{minimum } snf \text{ allowed}} \times 100,$$

or

$$100 - \frac{T.\ S.\ \text{found}}{\text{minimum } T.\ S,\ \text{allowed}} \times 100.$$

The added water calculated by this latter method is usually stated in the certificate of analysis as "at least . . . per cent."

Another formula for calculating the added water is

$$\text{Added water} = 100 - \frac{G+F}{34.5} \times 100$$ where $G$ = degrees of gravity or lactometer reading, and $F$ = the percentage of fat. The probable amount added may be obtained by substituting 36.0 for 34.5.

**Fat Abstraction.** The removal of cream is indicated by an abnormally low fat content and the minimum amount of fat abstraction may be calculated from the formula.

$$\text{Fat abstracted} = 100 - \frac{f}{F} \times 100$$ where $f$, and $F$, are the amounts of fat found in the sample and the minimum required by the standard, respectively. The probable amount removed may be obtained by substituting the average value for the month in which the sample is taken.

## Milk Standards

For the regulation of the sale of milk, various standards have been established which the mixed milk of a herd of cows might reasonably comply with, and it is, at least, this minimum quality that a purchaser expects to be supplied with. In England no specific standard has been adopted by statute but a standard of 3.0 per cent of fat and 8.5 per cent of solids-not-fat was adopted many years ago by the Society of Public Analysts as a guidance for analysts for milk that is of the nature, substance, and quality that might reasonably be demanded by the purchaser. The onus of proof regarding this contention, however, was upon the analyst, and it was not until 1901 that this was transferred to the vendor by an order of the Board of Agriculture which stated that milk containing less than 3.0 per cent of fat or 8.5 per cent of solids-not-fat shall be presumed to be not genuine *until the contrary is proved.* This has led to the " appeal to the cow " or the " stall " or " byre " test in which the cows are completely milked in the presence of a witness or witnesses and the milk afterwards analysed for comparison with the previous sample. If the results agree, the sample is to be regarded as genuine and to comply with the provisions of the Food and Drugs Act. It is obvious that great care should be taken in obtaining the test sample by insisting upon all the cows being thoroughly stripped of milk and, if possible, making the test on the same day of the week and at the same milking from which the first sample was obtained. Such a procedure evidently regards milk as the secretion of healthy cows without having regard to the breed, nature and quantity of food supply, and treatment of the cow, and this is apparently also the view of the Scottish High Court of Judiciary as expressed during the appeal of *Scott v. Jack.* Lord Johnston expressed the opinion that " milk in the sense of the statute is milk drawn from the cow, not milk in the process of formation in the chyle, in the blood, in the glands of the cow. . . ." This decision that milk is to be regarded as the secretion of healthy cattle leaves much to be

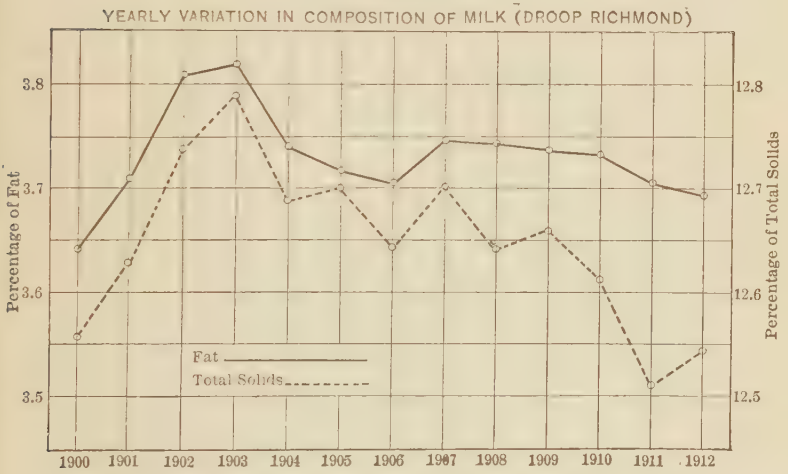
desired, as any breed may be used and the ration adjusted to secure quantity rather than quality and so lead to a diminution of both the average and minimum composition of the normal secretion.

The breeding of dairy cattle on scientific principles has led to the introduction of strains which secrete large quantities of milk of comparatively poor quality; the total weight of butter fat produced is at a maximum and when such milk is to be used for butter making this method of breeding must be regarded as legitimate and commended as a step forward in intensive breeding. When such produce is intended for sale as milk a very different view must be taken of such methods for, as regards the ultimate effect, there is no difference between this process and the deliberate addition of water to milk of superior quality. If milk is to be regarded as the secretion of cows, without additions or abstraction, it is evident that a premium is placed upon quantity regardless of quality, with the consequence that the water content of milk will become increasingly greater. It might be argued that such a course of reasoning is merely hypothetical inasmuch as the average composition of milk shows no definite tendency to deteriorate from decade to decade. Unfortunately there are comparatively few reliable records of data covering considerable periods. The records of the Aylesbury Dairy Co., London, as published by Droop Richmond, show that the milk supplied in 1912 was but very little different in composition to that supplied in 1900. The intervening period is marked by a rise in quality in 1902 and 1903 after which there is a steady decline. The results are set out in Diagram II.

The conditions in New York City present an entirely different aspect of this question. Prior to 1910 the standard demanded at least 12 per cent of total solids, but in that year the interests representing the Holstein breeders were strong enough to effect a reduction of the standard to 11.5 per cent. When this new standard became operative, no "quid pro quo" in the shape of a reduction in price was received by the consumer,

although the report of the Health Department states that "the reduction is a stimulus to adulteration and that the records of the department show that certain dealers, who, under the old law, were just within the standard of 12 per cent, are now selling milk, which repeated analyses have shown to be just within the lowered standard of 11.5 per cent of total solids." In this case it is evident that the quality of the milk supplied, by at least a portion of the producers, followed the standard,

DIAGRAM II




and it seems inevitable that the other producers will be driven to the adoption of similar measures by stress of competition.

In both the United States and Canada, milk standards are of an entirely different legal nature to those obtaining in Great Britain; the minimum limits of composition are clearly defined by ordinance or statute and admit of no appeal to the cow. These standards are to be regarded as specifications of what is required to be sold as milk and not the minimum quality that might reasonably be expected by the purchaser. This is equitable, as the purchaser, for a given price, should receive

an article of definite quality and not something that may be the minimum quality produced by natural variations. To achieve this, the dairyman must so grade his herd that the mixed milk will *at all times* comply with the standard. It may be argued that a rigid interpretation of a standard may inflict unnecessary hardship on producers by reducing what is usually but a comparatively small margin of profit, but it is surely preferable that the economic balance between producer and consumer should be adjusted by an increased price rather than by a deterioration in quality. The adjustment by price is understood by everyone whereas the maintenance of the balance by a reduction in quality is an invidious one only capable of being correctly appreciated by experts.

Rigid enforcement of standards is also necessary in the interests of dairymen in order to prevent unfair competition, as it is obviously unfair to allow some to breed for quantity and supply a quality which is, perhaps, only occasionally just below the standard, whilst others are supplying milk which is invariably above the standard. One typical example of this unfair competition which the author experienced was the case of producer X, who kept pure-bred Holsteins, which produced milk of the required standard, 12 per cent of total solids and 3.0 per cent fat, during the greater part of the year, but just failed to meet it during the season when the cows "freshened." An examination of the herd during this period showed that nine cows, out of the 22 head comprising the herd, secreted a low quality of milk and were giving an abnormally large quantity, one cow producing as much as $7\frac{1}{2}$ gallons per day. This producer had an obvious advantage over others whose herds were graded with Ayrshires and other breeds giving a higher quality but a smaller quantity.

The standards prescribed in various countries show but small differences; those prevailing in States, provinces and cities, which have power to make local regulations unfortunately show larger variations and these often conflict with those of contiguous authorities. Table XXXIV gives a fairly

complete list of the standards for milk and cream obtaining in English-speaking countries.

## Table XXXIV

### MILK AND CREAM STANDARDS

| Country, State or Province. | Milk. | | | Skim Milk. | Cream. | |
|---|---|---|---|---|---|---|
| | Total Solids. | Fat. | Solids-Not-fat. | Solids-Not-fat. | Fat. | |
| Great Britain | ..... | 3.00 | 8.50 | | | |
| *Australia.* | | | | | "Full" | "Half" |
| New South Wales | ..... | 3.20 | 8.50 | 8.80 | 35.0 | 25.0 |
| | | | | | "Double" | "Single" |
| South Australia | 12.00 | 3.25 | 8.50 | 8.80 | 35.0 | 25.0 |
| | | | | | "Cream" | "Reduced Cream" |
| Victoria | 12.00 | 3.50 | 8.50 | 8.80 | 35.0 | 25.0 |
| | | | | | Cream. | |
| Queensland | 12.00 | 3.30 | 8.50 | 8.80 | 35.0 | |
| | | | | | "Double" | "Single" |
| Western Australia | 11.70 | 3.20 | 8.50 | 8.80 | 35.0 | 25.0 |
| | | | | | Cream. | |
| Tasmania | 12.00 | 3.30 | 8.50 | 8.80 | 35.0 | |
| *Canada.* Dominion: | ..... | 3.25 | 8.50 | 8.50 | 18.0 | |
| Alberta | 12.00 | 3.00 | 9.00 | 8.50 | | |
| British Columbia | 11.75 | 3.25 | 8.50 | | | |
| Manitoba | ..... | 3.25 | 8.50 | 8.50 | 18.0 | |
| New Brunswick | No Standards | | | | | |
| Nova Scotia | No Standards | | | | | |
| Ontario | 12.00 | 3.00 | | | 18.0 | |
| Quebec | 12.00 | 3.00 | 9.00 | ..... | 16.0 | |
| Saskatchewan | 12.00 | 3.50 | ..... | ..... | 20.0 | |
| *South Africa* | ..... | 3.00 | 8.50 | ..... | 25.0 | |
| *New Zealand* | ..... | 3.25 | 8.50 | 8.80 | 25.0<br>40.0 | |
| *India.* | | | | | | |
| Calcutta | 11.50 | 3.00 | 8.50 | | | |
| Bombay | 12.00 | 3.50 | 8.50 | | | |

## TABLE XXXIV—(*Continued*)

### MILK AND CREAM STANDARDS

| Country, State or Province. | MILK. | | | SKIM MILK. | CREAM. |
|---|---|---|---|---|---|
| | Total Solids. | Fat. | Solids-Not-fat. | Solids-Not-fat. | Fat. |
| United States. Federal. | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| California | ..... | 3.00 | 8.50 | 8.80 | 18.0 |
| Colorado | ..... | 3.00 | ..... | ..... | 16.0 |
| Connecticut | 11.75 | 3.25 | 8.50 | ..... | 16.0 |
| District of Columbia | 12.50 | 3.50 | 9.00 | 9.30 | 20.0 |
| Florida | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Georgia | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Idaho | 11.20 | 3.00 | 8.00 | 9.30 | 18.0 |
| Illinois | ..... | 3.00 | 8.50 | 9.25 | 18.0 |
| Indiana | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Iowa | 12.00 | 3.00 | ..... | ..... | 16.0 |
| Kansas | ..... | 3.25 | ..... | ..... | 18.0 |
| Kentucky | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Louisiana | .75 | 3.50 | 8.50 | 8.00 | |
| Maine | 11.75 | 3.25 | 8.50 | ..... | 18.0 |
| Maryland | 12.50 | 3.50 | ..... | 9.25 | 18.0 |
| Massachusetts | 12.15 | 3.35 | ..... | 9.30 | 15.0 |
| Michigan | 12.50 | 3.00 | ..... | ..... | 18.0 |
| Minnesota | 13.00 | 3.25 | 9.75 | ..... | 20.0 |
| Missouri | 12.00 | 3.25 | 8.75 | 9.25 | 18.0 |
| Montana | 11.75 | 3.25 | 8.50 | ..... | 20.0 |
| Nebraska | ..... | 3.00 | ..... | ..... | 18.0 |
| New Hampshire | 12.00 | ..... | 8.50 | ..... | 18.0 |
| New Jersey | 11.50 | 3.00 | ..... | 9.25 | 16.0 |
| Nevada | 11.75 | 3.25 | 8.50 | 9.25 | 18.0 |
| New York | 11.50 | 3.00 | ..... | ..... | 18.0 |
| North Carolina | 11.75 | 3.25 | 8.50 | 9.25 | 18.0 |
| North Dakota | 12.00 | 3.00 | ..... | ..... | 15.0 |
| Ohio | 12.00 | 3.00 | | | |
| Oregon | 11.70 | 3.20 | 8.50 | ..... | 18.0 |
| Pennsylvania | 12.00 | 3.25 | 8.50 | ..... | 18.0 |
| Rhode Island | 12.00 | 3.50 | | | |
| South Dakota | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Tennessee | 12.00 | 3.50 | 8.50 | 9.00 | 20.0 |
| Texas | 12.00 | 3.25 | 8.50 | 9.25 | 18.0 |
| Utah | 12.00 | 3.20 | 8.50 | ..... | 18.0 |
| Vermont | 11.75 | 3.25 | 8.50 | 9.25 | 18.0 |
| Virginia | ..... | 3.25 | 8.50 | 9.25 | 18.0 |
| Washington | 12.00 | 3.25 | 8.75 | 9.30 | 18.0 |
| Wisconsin | ..... | 3.00 | 8.50 | 9.00 | 18.0 |

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## CHAPTER III

### CHEMICAL EXAMINATION

Although the extent of the chemical examination of milk required in public health work is usually confined to the determination of the fat and total solids and the detection of preservatives, a brief description of reliable methods for the estimation of other constituents will also be given in this chapter as they are invaluable for the correct diagnosis of sophistication.

As the great majority of ordinances and statutes regulating the sale of milk contain no reference to constituents other than fat and total solids, these will be considered first.

**Estimation of Fat.** The various methods introduced for the determination of fat in milk may be divided into three groups.

(1) Volumetric estimation of the fat brought to the surface by centrifugal force after liberation by the addition of chemicals.

(2) Ethereal extraction of the fat liberated by the addition of chemicals.

(3) Ethereal extraction of the dried milk.

The methods which comprise the second group, though invaluable for dealing with milk products, are not in general use for the examination of fresh milk and will not be given in detail.

The mechanical methods of group one are now in almost universal use and are capable, in practised hands, of yielding accurate results. The three chief mechanical methods are the Leffmann-Beam, Babcock, and Gerber. In England, the Leffman-Beam and the Gerber are almost exclusively used whilst in America, although both the Babcock and Gerber processes are official, the former is more generally employed.

**Leffmann-Beam Process.** 15 c.cms. of the sample are transferred by means of a pipette into a flat-bottomed bottle provided with a narrow neck graduated into 80 divisions, 10 of which correspond to 1 per cent of fat by weight. 9 c.cms. of concentrated commercial sulphuric acid are then added in three portions with thorough admixture after each, and finally, 3 c.cms. of a mixture of equal volumes of concentrated hydrochloric acid and amyl alcohol. After shaking, the bottle is filled to the zero mark with hot dilute sulphuric acid (1 in 2) and whirled in the centrifuge for 3 to 4 minutes. The fat rises to the top of the liquid as a yellowish coloured layer and the percentage is read off by deducting the reading at the junction of the fat and acid from the reading at the extreme top of the fat, not the bottom of the meniscus.

**Babcock Method.** This method differs from the Leffmann-Beam process in but a few details. The bottle neck is divided into 50 divisions each representing 0.2 per cent of fat by weight of the 17.6 c.cms. employed. The procedure is as follows: the milk having been placed in the bottle 17.5 c.cms. of commercial sulphuric acid are gradually added with constant agitation until the caseinogen is dissolved. The bottle is then placed in a centrifuge and whirled for four minutes at 690 to 1200 revolutions per minute, according to the diameter of the machine; hot water is added until the bottle is filled to the lower end of the neck, whirled for one minute, then filled to the zero mark with hot water and whirled for one further minute to bring the fat layer into the graduated neck. The percentage of fat is then read off as in the Leffman-Beam method, care being taken that all readings are made between 130° and 150° F. when the fat is quite liquid. The author has found that the indistinct line of demarkation between the fat and the acid occasionally found with this process can be obviated by the addition of 1 c.cm. of amyl alcohol after the addition of the acid.

**Gerber Method.** This differs from the modified Babcock described only in the size and type of bottle, and quantities of acid and milk employed. 11 c.cms. of milk, 1 c.cm. of amyl

alcohol, and 10 c.cms. of sulphuric acid are mixed in the usual way, rotated for three minutes, then immersed in a water bath at 140° F. for a minute and the percentage of fat read off on the graduated neck.

Skim milk is treated exactly as ordinary milk except in the Gerber process in which two to three minutes shaking are required previous to whirling and a longer period is given in the water bath to bring the temperature to 140° F.

For cream, special bottles are provided in the Babcock method, but the ordinary ones may be used, as in the Leffmann-Beam method, with a reduced quantity of sample. An appropriate weight of the sample is washed into the bottle with sufficient water to bring the total volume to the normal volume of the bottle, and the determination carried out as in the case of milk. The result is multiplied by the ratio of the normal weight of the method (Leffmann-Beam 15.5 grms., Babcock 18.0 grms.) to the weight of the sample taken. In the Gerber process (normal weight 11.35 grms) 0.5 gram of cream, 6 c.cms. of hot water, 1 c.cm. of amyl alcohol, and 6.5 c.cms. of acid are used with a further addition of 6 c.cms. of hot water previous to rotation.

### Gravimetric Methods

**Gottlieb's Method.** In this method, which is probably the best known one of group two, the caseinogen is dissolved in ammonia and the liquid then extracted with ether and petroleum ether. The solution of fat is evaporated and the residue weighed. For further details of this process Richmond's Dairy Chemistry (Chas. Griffin & Co., London, 1914) should be consulted.

**Adam's Method.** 5 grams of milk are weighed out in a porcelain or glass dish and absorbed on a coil of fat free paper (special strips of fat-free paper are manufactured for this purpose by various firms). The dish and coil are placed in the water oven until thoroughly dry when the coil is placed in a Sohxlet extraction cone and the residue in the dish extracted

several times with absolute ether. The ether so used is poured over the coil and cone, previously placed in the extraction apparatus, and, after the volume of solvent has been increased, the apparatus is connected with a condenser and heated in a water bath at about 45° C. After four or five hours extraction the ether is distilled off and the fat dried to constant weight. The removal of the ether is facilitated by drawing a current of air through the flask by means of a vacuum pump. It is necessary that the ether used in this process should be perfectly dry, as otherwise small quantities of milk sugar and salts are extracted with the fat.

This is the official method of the Society of Public Analysts of Great Britain and one of the official methods of the American Official Association of Agricultural Chemists.

**Total Solids.** These may be determined either directly by drying to constant weight or indirectly by calculation from the fat content and the specific gravity.

**Direct Method.** Five grams of milk are weighed into a shallow platinum or quartz dish and after all visible liquid has been driven off on the water bath, the dish and contents are dried to constant weight in a steam oven. Ignited sand or asbestos may be used to facilitate the drying process.

**Ash.** The residue from the determination of the total solids may be ignited at a low temperature until white and the residue weighed, or a fresh portion of 20 c.cms. evaporated with the addition of 6 c.cms. of nitric acid, and ignited until free from carbon at a temperature just below redness. The former method is the more convenient and the latter the more accurate one.

**Specific Gravity.** This is determined either by a lactometer, a Westphal balance, or the ordinary specific gravity bottle. The lactometer method is the simplest and quickest, but, owing to the comparatively short space occupied by each graduation (usually 1°) and the opalescence of the liquid the degree of accuracy obtained is low.

The gravity is usually expressed as the excess weight of

1000 c.cms. of milk at 60° F. over an equal volume of water at the same temperature. Thus, a Specific Gravity of 1032.2 (water = 1000) is usually expressed as 32.2 or, 32.2° lactometer scale.

Lactometers indicate the specific gravity at a temperature of 60° F. and it is, therefore, necessary to either bring the sample to this temperature or to correct the reading. It is much more convenient to ascertain the temperature of the sample immediately before taking the specific gravity and to correct this result to 60° F. by means of Table LXVIII, which will be found in the appendix.

It is important that the specific gravity of milk should not be determined within a short period of milking as, during the first four hours, there is a decided increase often amounting to 1 to 1.5° (Recknagel's phenomenon). The gravity should also never be taken immediately after violent agitation of the sample as the air entrapped by the fat globules during such a process may lead to serious errors. If violent agitation is necessary for any purpose, it is advisable to allow the sample to remain quiescent for two hours before proceeding with the specific gravity determination. No attempt should be made to take the specific gravity of a sample that has commenced to curdle.

**Total Solids, by Calculation.** As the fatty and non-fatty portions of milk are comparatively constant in composition, it is evident that the specific gravity of milk can be calculated from the percentages of these constituents. Fat tends to reduce the gravity, and non-fatty solids to increase it. Hehner and Richmond found that the following formula expressed with a fair degree of accuracy the quantitative relation between these constituents:

$$F = 0.859\ T.\ S. - 0.2186G.$$

Where $F$ = percentage of fat, $T.\ S.$ the percentage of total solids and $G$ the specific gravity expressed as mentioned above. From this formula $T.\ S. = 1.164F + 0.2546G$.

A simplified form of this formula that has come into general

use is $T.S. = 1.2F + 0.25G$. This is, with very slight modifications, the basis of Babcock's tables which are official in America. Richmond now prefers the formula $T.S. = 1.2F + 0.25G + 0.14$ and this was used in the preparation of the slide rule which so greatly facilitates the calculation of the total solids from the fat and specific gravity determinations. It is advisable to remember that the differences between the results obtained by use of the various formulæ are within the limits of experimental error and that a direct determination should be made when great accuracy is required.

Richmond's and Babcock's tables are given in the appendix on pages 210–213.

**Solids Not-fat.** These are estimated by deducting the percentage of fat from that of the total solids or they may be calculated directly from the gravity and the percentage of fat.

**Milk Sugar.** Milk Sugar, or Lactose, may be estimated by either the polarimetric, volumetric, or gravimetric methods. When a polarimeter is available, this method is almost invariably employed as but little time is required for the examination of several samples. In the absence of this instrument, and when only occasional determinations are required, the gravimetric method should be used.

*Polarimetric Methods.* These are based upon the examination of the milk serum in a polariscope after the separation of the fat and proteids. A solution of mercuric nitrate, prepared by dissolving mercury in twice its weight of nitric acid (1.42) and diluting with an equal volume of water, is the most suitable reagent for this purpose. As the removal of proteids and fat reduce the volume of the lactose containing solution, it is necessary to correct the readings for the percentages of these constituents, but Richmond and Boseley (Dairy Chemistry) point out that these calculations can be simplified by the use of the following method.

To 100 c.cms. of milk add

(*a*) A quantity of water in c.cms. equal to $\frac{1}{10}$ the lactometer reading or excess gravity over 1000.

(*b*) A quantity of water in c.cms. equal to the fat×1.11.

(*c*) A quantity of water in c.cms. to reduce the scale readings to percentages of milk sugar.

(*d*) 3 c.cms. of acid mercuric nitrate.

After thorough agitation, filter through dry papers and polarise the filtrate. The percentage of milk sugar can be read off directly in scale readings.

The values of (*c*) are:

(*a*) For polariscopes reading angular degrees.

With 198.4 mm. tube 10.0 c.cms.
With 200 mm. tube 10.85 c.cms.
With 500 mm. tube 10.85 c.cms. (divide readings by 2.5).

(*b*) For the Laurent sugar scale (100° = 21.67 angular degs.)

With 200 mm. tubes 2.33 c.cms. (divide readings by 5)
With 400 mm. tubes 2.33 c.cms. (divide readings by 10).
With 500 mm. tubes 2.33 c.cms. (divide readings by 12.5)

(*c*) For the Ventzke scale (100° = 34.64 angular degrees).

With 200 mm. tube 6.65 c.cms. (divide readings by 3).
With 400 mm. tube 6.65 c.cms. (divide readings by 6).
With 500 mm. tube 6.65 c.cms. (divide readings by 7.5).

*Gravimetric Method.* Dilute 25 c.cms. of milk with 400 c.cms. of water in a 500 c.cm. flask, add 10 c.cms. of No. 1, Fehling solution and 4.4 c.cms. of N·NaOH solution; make up to 500 c.cms., shake, and filter through a dry paper. The filtrate should be acid and contain copper in solution. Place 25 c.cms. each of Fehling's solutions Nos. 1 and 2 in a beaker and heat to the boiling point. When boiling briskly add 100 c.cms. of the milk serum and boil for six minutes. Filter immediately through asbestos, supported by a platinum cone in a hard glass filtering tube, with the aid of a suction pump, wash thoroughly with boiling water and finally with alcohol followed by ether. After drying, connect the tube with an apparatus for supplying a continuous current of hydrogen and gently heat until the cuprous oxide is completely reduced to the

metallic state. Cool in an atmosphere of hydrogen and weigh. The weight of copper is calculated to lactose from Table LXXI in the appendix.

The weight of lactose×20 gives the percentage per 100 c.cms. of sample. As an alternative method of weighing the reduced oxide, a Gooch crucible may be used in which a layer of asbestos about one-quarter of an inch in thickness has been placed. Wash the asbestos thoroughly with hot water and then with 10 c.cms. of alcohol followed by 10 c.cms. of ether. Dry for thirty minutes in the steam oven and weigh. The precipitate of cuprous oxide is collected as above, washed with water, treated with 10 c.cms. of alcohol and ether, successively, and dried for thirty minutes at 100° C. The weight of $Cu_2O$ multiplied by 0.8883 gives the weight of metallic copper.

### Proteids

**Total Proteids.** 5 gms. of milk are placed in a Kjeldahl flask of about 150 c.cms. capacity and 20 c.cms. of pure conc. sulphuric acid added. The mixture is heated over a small flame until excessive frothing has ceased, and after cooling, 8–10 grms. of acid potassium sulphate and a drop of mercury are added. After placing a sealed funnel containing water in the mouth of the flask to prevent excessive evaporation, the contents of the flask are gradually heated and the flame slightly increased as frothing ceases. When the liquid becomes colourless the flask is allowed to cool and the contents washed with the aid of distilled water into a flask. This flask should be provided with a stopper having two holes, one containing a trapped bulb tube connected with a water condenser, and the other a tapped funnel reaching almost to the bottom of the flask. After the contents of the Kjeldahl flask have been transferred, a few pieces of pumice, unglazed porcelain, or granulated zinc, are added to prevent bumping and the distillation apparatus connected up with the outlet of the condenser dipping into a beaker containing 50 c.cms. of $\frac{N}{10}$ acid. Through the funnel add 100 c.cms.

of 30 per cent caustic soda, followed by 10 c.cms. of a 10 per cent solution of potassium sulphide. The flame is placed under the flask, and the distillation continued until about 200 c.cms. have passed over. Before taking away the flame, the tap of the funnel should be opened to prevent creating a partial vacuum and so drawing back the distillate into the flask. The end of the condenser is washed with water, and the washings mixed with the distillate which is finally titrated with $\frac{N}{10}$ caustic alkali using sensitive methyl orange or, preferably, methyl red as the indicator. Each c.cm. of $\frac{N}{10}$ acid neutralised = 0.0014 grm. nitrogen or 0.028 per cent of nitrogen when 5 grms. of milk are used. The percentage of nitrogen multiplied by 6.38 gives the percentage of total proteids.

In all determinations of nitrogen by the above method, it is essential that a blank determination should be made on all the reagents and this amount deducted from all subsequent results.

**Caseinogen.** Dilute 10 gms. of the sample with about 90 c.cms. of water at 40° to 42° C. and add at once 1.5 c.cm. of a 10 per cent acetic acid solution. Stir with a glass rod and allow to stand for about five minutes. Decant on to a wet filter, wash several times with cold water by decantation and then transfer the precipitate completely to the filter. Wash once or twice with cold water. If the filtrate is not bright it should be refiltered until that condition is attained. The nitrogen in the precipitate is then estimated as above by the Kjeldahl method. The percentage of nitrogen multiplied by 6.38 gives the percentage of caseinogen. This method is only applicable to fresh milk.

**Albumin.** The filtrate from the precipitation of caseinogen is first exactly neutralised with caustic alkali and then acidified by the addition of 0.3 c.cm. of a 10 per cent solution of acetic acid. After heating to boiling over a flame, the precipitate is digested on the water bath for fifteen minutes. The liquid is filtered through paper, the precipitate washed and finally used

for a nitrogen determination by the Kjeldahl method. Nitrogen $\times 6.38 =$ Albumin.

**Total Acidity.** Lactic Acid. 10 c.cms. of milk are placed in a white porcelain basin, a few drops of phenolphthalein solution added and titrated with $\frac{N}{10}$ alkali until a faint pink colour is obtained. As the acidity of fresh milk is chiefly due to phosphates, the expression of the acidity in terms of lactic acid is somewhat misleading, although this is often done, 1 c.cm. of $\frac{N}{10}$ alkali being equivalent to 0.009 grm. lactic acid. It is preferable to express the acidity in degrees, i.e., the number of cubic centimeters of normal alkali required for the neutralisation of 1 litre of milk. The number of cubic centimeters of $\frac{N}{10}$ alkali required for the neutralisation of 10 c.cms. of milk, multiplied by 10 gives the required result in degrees. It is unfortunate that in Germany the same term is used for a unit having a very different value. The Sohxlet-Henkel degree usually used throughout Germany is exactly 2.5 times greater than the degree used in England and America.

**Aldehyde Value.** Richmond and Miller's modification (Richmond's Dairy Chemistry) of Steinegger's method is as follows: 10 c.cms. of milk are made neutral to phenolphthalein with $\frac{N}{10}$ strontia, 2 c.cms. of 40 per cent formaldehyde added, and again titrated to the same degree of neutrality. The amount of the second addition of alkali less the amount required for the neutralisation of the formaldehyde added (previously determined), multiplied by 10 gives the aldehyde value.

This method is dependent upon the fact that the proteid radicle is quantitatively converted to an acid by the aldehyde. Richmond states that the strontia aldehyde figure is 1.1 times greater than that given with $\frac{N}{10}$ soda and that the former value multiplied by 0.170 will give a close approximation to the total

proteids. It is also pointed out that as caseinogen and albumin do not give the same aldehyde value, the factor is only applicable when the ratio of caseinogen to albumin is normal.

**Mineral Constituents.** The estimation of the mineral constituents in milk is but infrequently required in connection with public health work but on these occasions, the following method, due to Droop Richmond, will be found advantageous as it secures fairly accurate results with a minimum expenditure of time and labour.

Fifty grams of milk are evaporated and charred to a black ash: the mass is extracted with hot water and filtered, the insoluble portion, together with the paper (after washing) being ignited until white; this gives the insoluble ash. Evaporation of the filtrate and cautious heating gives the weight of the soluble ash.

The soluble ash, after solution in water, is made up to a known volume and aliquot portions used for the determination of the alkalinity by titration with $\frac{N}{10}$ acid with methyl orange as indicator, and chlorine by titration with $\frac{N}{10}$ silver nitrate, using potassium chromate as indicator. 1 c.cm. of $\frac{N}{10}$ acid $= 0.0031$ grm. $Na_2O$ and 1 c.cm. $\frac{N}{10}$ $AgNO_3 = 0.00355$ grm. Cl.

The insoluble ash is dissolved in a slight excess of dilute hydrochloric acid, and the solution (nearly neutralised if necessary) heated to boiling; a cold saturated solution of ammonium oxalate is dropped in slowly until further addition produces no further precipitate. After standing at least two hours, the precipitate is filtered off, washed, and ignited at a low temperature to convert the oxalate into carbonate; it is advisable to moisten the ignited precipitate with ammonium carbonate solution and reignite at a very low temperature. The precipitate, after weighing, is dissolved in dilute hydrochloric acid, keeping the bulk small, ammonia is added to alkaline reaction,

and the small precipitate of calcium phosphate collected, ignited, and weighed. Its weight is subtracted from the previous weight, and the difference gives the weight of calcium carbonate, which, multiplied by 0.4, gives the calcium, or by 0.56, the lime (CaO) contained in it; the weight of calcium phosphate multiplied by 0.3871 gives the calcium (Ca), or by 0.5419, the lime (CaO) contained in it. The total calcium or lime is the sum of the two.

The filtrate is made strongly ammoniacal by the addition of strong ammonia (0.880) and allowed to stand twenty-four hours. The precipitated magnesium ammonium phosphate is filtered off, washed with dilute ammonia, ignited, and the magnesium pyrophosphate ($Mg_2P_2O_7$) weighed. Its weight multiplied by 0.2162 will give the magnesium (Mg), or by 0.3604, the magnesia (MgO) contained in it.

To the filtrate from this, magnesia mixture is added, and the precipitate, after standing twenty-four hours, is treated as above. From the total weight of the two quantities of magnesium pyrophosphate, the phosphoric anhydride is calculated by multiplying by 0.6396; to this is added the phosphoric anhydride in the calcium phosphate, calculated by multiplying the weight by 0.4581. This method takes no account of the traces of iron present, which are precipitated with the calcium phosphate and the magnesium-ammonium phosphate. If desired, this may be estimated by dissolving the precipitate of calcium phosphate and the first magnesium-ammonium phosphate precipitate in dilute hydrochloric acid, and determining the iron colorimetrically as thiocyanate.

To estimate alkalies, another portion of milk is ignited as before, and the total ash dissolved in dilute hydrochloric acid and boiled; a few drops of barium chloride solution, containing not more than 0.1 grm. of barium to 100 grms. of milk are added, and the boiling continued for some minutes. After some hours, the precipitate of barium sulphate is filtered off, washed, ignited, and weighed; its weight multiplied by 0.3433, will give the sulphuric anhydride ($SO_3$) in the milk. If an excess

of barium chloride has been added, a little phosphoric acid, or ammonium phosphate, may now be added to the filtrate, although it is not necessary if the quantity of barium chloride given above has been employed. A quantity of ferric chloride solution, sufficient to colour the solution brown, is added and the filtrate made alkaline with ammonia. After boiling, the precipitate is filtered off and well washed: the filtrate is evaporated and cautiously ignited: this weight represents the alkaline chlorides. When the residue is dissolved in hot water, the solution should be perfectly clear; if this be not so, a little ammonium carbonate solution is added, the liquid evaporated to dryness and the residue cautiously ignited; the residue is again taken up with water, the solution filtered and evaporated, and the residue cautiously ignited and weighed. This purification of the mixed alkaline chlorides is often found necessary and it is essential, in order that accurate results may be obtained, that the process should be carried out with great care, always bearing in mind that alkaline chlorides are volatilised at comparatively low temperatures.

The chlorine in the mixed chlorides may be estimated by titration with $\frac{N}{10}$ silver nitrate, using potassium chromate as indicator. Each cubic centimeter of $\frac{N}{10}$ $AgNO_3$ is equivalent to 0.00355 grm. chlorine. The potassium and sodium are calculated from the formulæ.

$$\text{The weight of sodium} = 2.997C - 1.4254W,$$

$$\text{The weight of potassium} = 2.4254W - 3.987C.$$

in which $W$ = the weight of the mixed alkaline chlorides,

and $C$ = the weight of chlorine therein.

**Examination of Milk Serum.** As the fat and proteids are the most variable constituents of milk, an examination of the milk serum often affords valuable assistance in determining

whether a sample is adulterated by the addition of water, or is merely abnormal in composition. The principal constituents of the serum are milk sugar and mineral matter, and a determination of these on the milk direct affords the same evidence as an indirect examination of the serum, but as the latter can be

TABLE XXXV

RELATION OF REFRACTIVE INDEX TO SPECIFIC GRAVITY

(LYTHGOE)

| Scale Reading Immersion Refractometer. 20° C. | $n_D$ 20° C. | Specific Gravity. $\frac{15°}{15°}$ |
|---|---|---|
| 28.0 | 1.33820 | 1.0149 |
| 29.0 | 1.33861 | 1.0160 |
| 30.0 | 1.33896 | 1.0170 |
| 31.0 | 1.33934 | 1.0180 |
| 32.0 | 1.33972 | 1.0190 |
| 33.0 | 1.34010 | 1.0200 |
| 34.0 | 1.34048 | 1.0211 |
| 35.0 | 1.34086 | 1.0221 |
| 36.0 | 1.34124 | 1.0231 |
| 37.0 | 1.34162 | 1.0242 |
| 38.0 | 1.34199 | 1.0252 |
| 39.0 | 1.34237 | 1.0262 |
| 40.0 | 1.34275 | 1.0273 |
| 41.0 | 1.34313 | 1.0283 |
| 42.0 | 1.34350 | 1.0293 |
| 43.0 | 1.34388 | 1.0303 |
| 44.0 | 1.34426 | 1.0313 |
| 45.0 | 1.34463 | 1.0323 |

performed more expeditiously, it is often included in the routine examination of milk. The serum is prepared by adding 2 c.cms. of 25 per cent acetic acid (Sp. Gr. 1.035) to 100 c.cms. of sample at a temperature of 20° C., covering with a watch-glass and heating to 70° C. for twenty minutes. After cooling in ice water for ten minutes, the curd is separated by filtration

through paper and 35 c.cms. of the filtrate, which should be bright, are transferred to one of the beakers which accompany the Zeiss immersion refractometer. The refraction is then determined at exactly 20.0° C. A reading between 39.0 and 40.0 is suspicious whilst one less than 39.0 indicates the addition of water.

Lythgoe[1] after determining the value of $K$ in the Lorenz and Lorentz formula

$$\frac{n^2-1}{n^2+2}\cdot\frac{d}{1}=K,$$

which expresses the relation between the refractive index ($n$) and the specific gravity ($d$), has calculated the values of $d$ for the various scale readings of the immersion refractometer, and in the absence of this instrument, the specific gravity determination will achieve the same object after reference to Lythgoe's table. (Table XXXV, p. 79.)

### Detection and Estimation of Preservatives

The addition of preservatives to milk is usually absolutely prohibited because it has been found perfectly feasible to market this product in a sound condition without their use. No legitimate excuse, therefore, for the addition of any substance which retards or inhibits bacterial development. Although the exigencies of certain branches of trade in milk products have, in some cases, led to the adoption of regulations which permit the addition of certain specified preservatives in quantities not exceeding a specified limit, this practice should not be encouraged, for, until it can be proved beyond reasonable doubt that such preservatives are non-toxic, the public should be safeguarded against these substances: public health should be paramount to commercial interests and not sacrificed to them. Unfortunately many statutes regarding the sophistication of foodstuffs are even yet so framed as to place the onus of proof as to damage to health upon the prosecutor and so give the defendant the benefit of all doubts that may exist, but it is pleasing to note that these are decreasing and that the present

tendency is to prohibit the entire use of particular preservatives and to restrict them generally.

The preservatives in most general use are boric acid, borax, or mixtures of these two, and formaldehyde. For milk the last-mentioned is the favourite owing to its potency and general convenience. The presence of boric acid or borax is allowed in cream in England when declared on the label attached to the container and in quantities not exceeding 0.25 per cent when calculated as boric acid. Harden has shown that the addition of an alkali (7 grms. of $Na_2O$ per 100 grms. of boric acid) increases the efficiency of boric acid as a preservative, and it is now customary to employ such a mixture for the preservation of cream. Such mixtures also contain cane sugar or traces of saccharin, the object of which is to mask incipient sourness.

**Formaldehyde.** Formaldehyde may be detected by any of the following tests, but on account of its reliability and delicacy, the author recommends the Shrewsbury and Knapp process.

*Hehner Method.* About 10 c.cms. of sample are placed in a test tube and concentrated commercial sulphuric poured carefully down the side so as to form a layer beneath the milk. In the presence of formaldehyde, a violet ring is formed at the junction of the two liquids. Richmond and Boseley modified the test by adding an equal volume of water to the milk and using acid of 90 to 94 per cent strength. One part in 200,000 produces a violet colouration which is permanent for several days. In the absence of formaldehyde, a greenish ring is produced and a brick-red colouration in the acid layer.

Leonard [2] points out that the presence of a mild oxidising agent is essential for the success of this test and that such an agent, preferably a trace of ferric chloride, must be added if pure acid is used. Droop Richmond [3] points out that the test is dependent upon the reaction of formaldehyde with the tryptophane of the caseinogen and that other aldehydes, e.g., vanillin, give similar reactions.

*Hydrochloric Acid Test.* 10 c.cms. of commercial hydro-

chloric acid, containing 0.2 grm. of ferric chloride per litre, are added to 5 c.cms. of milk in a porcelain basin and the mixture heated to boiling with constant stirring. The presence of formaldehyde is indicated by a violet colouration.

*Shrewsbury and Knapp Test.*[4] The reagent for this test consists of a freshly prepared mixture of pure concentrated hydrochloric acid with 0.1 per cent of pure nitric acid. 5 c.cms. of the sample are placed in a test tube and vigourously shaken with 10 c.cms. of the reagent, the mixture is heated in a water bath to 50° C. for ten minutes and finally rapidly cooled to about 15° C. A violet colouration denotes the presence of formaldehyde, and a rose pink colouration, its absence. The depth of the colouration, between 0.2 and 6 parts per million, is approximately proportional to the amount of formaldehyde present, so that this method may also be used for the estimation of the preservative. When the amount exceeds six parts per million, the milk should be suitably diluted.

**Estimation of Formaldehyde.** In addition to the method previously mentioned, various others have been devised for the estimation of formaldehyde, but not one as yet can be relied upon to give accurate results. Most of these are based upon the volatilisation of the aldehyde by distillation of an acid solution, and subsequent volumetric estimation. Probably the most useful is the following. To 100 c.cms. of sample contained in a 500 c.cm. Kjeldahl flask add 1 c.cm. of 1 : 3 sulphuric acid and distil over 20 c.cms. (care is necessary if frothing is to be avoided). The formaldehyde in the distillate, amounting to approximately one-third of the total, is estimated iodometrically. 25 c.cms. of $\frac{N}{10}$ iodine solution are added to the distillate and normal caustic soda is added, drop by drop, until the liquid becomes a clear yellow. After standing for fifteen minutes, dilute sulphuric acid is added in excess to liberate the uncombined iodine. The solution is then titrated with $\frac{N}{10}$ sodium thiosulphate, using a starch solution as the indicator in the end

reaction. Each cubic centimetre of $\frac{N}{10}$ iodine solution absorbed equals 0.0015 grm. of formaldehyde.

Monier-Williams, in a report to the Local Government Board, states that a preservative is on the market which contains a nitrite in addition to formaldehyde: the nitrite masks the usual reactions but its effect may be destroyed by the addition of a little urea.

**Boric Acid and Borates.** These may be detected by adding a few cubic centimetres of normal alkali to not less than 10 cubic centimetres of milk and evaporating to dryness over a small flame. The flame is increased until a black ash results: this is acidified with a few drops of hydrochloric acid. After lixiviation with a few cubic centimetres of hot water, the ash is removed by filtration through paper. A turmeric paper is placed in the filtrate in such a manner that only a portion of it can be wetted, and the liquid evaporated to dryness. A reddish-brown colouration of the wetted portion, due to the formation of rosocyanin, indicates the presence of boron compounds. A drop of caustic soda changes the colouration to various shades of green and purple which can be restored to the original colour by the addition of hydrochloric acid.

A useful routine method for the detection of boron compounds consists in heating about 10 c.cms. of milk in a porcelain dish with a few cubic centimetres of methyl alcohol and a few drops of tincture of turmeric. The heating is conveniently carried out in a water bath and the presence of boron compounds is indicated by the formation of a reddish ring round the basin.

The *estimation* of boron compounds is most conveniently carried out by Thomson's method.[5] One or two cubic centimetres of N·NaOH are added to 100 c.cms. of milk and the whole evaporated to dryness in a platinum dish. The residue is ignited to a black ash, heated with 20 c.cms. of water and concentrated hydrochloric acid added, drop by drop, until frothing ceases. The solution containing the carbonaceous

matter is washed with a few cubic centimetres of water into a 100 c.cm. flask and 0.5 grm. dry calcium chloride added. After the addition of a few drops of phenolphthalein, a 10 per cent solution of caustic soda is added until a faint pink colour persists and finally 25 c.cms. of lime water. The object of this is to precipitate the phosphates as calcium phosphate. Make up the volume to 100 c.cms., mix thoroughly and filter through a dry paper. To 50 c.cms. of the filtrate add N. sulphuric acid until just colourless, then add a few drops methyl orange and continue the titration until the yellow colour changes to pink. $\frac{N}{10}$ soda is now added until the reaction is just alkaline and the liquid boiled to expel the carbonic acid liberated. The solution is cooled, a few drops of phenolphthalein solution and sufficient neutral glycerine to amount to 40 per cent of the total volume is added. The solution is finally titrated with $\frac{N}{10}$ soda until a permanent pink colouration is produced. Each cubic centimetre of $\frac{N}{10}$ soda equals 0.0062 grm. of boric acid.

**Benzoic Acid.** The proteids are precipitated by the addition of 5 c.cms. of dilute hydrochloric acid and shaking: then shake with several portions of ether, taking care to avoid the formation of an emulsion. If this should occur, resort must be made to a centrifuge in order to separate it. The ethereal extract containing the benzoic acid and fat is shaken with water, rendered alkaline by the addition of ammonia, and the aqueous extract evaporated nearly to dryness. After all the ammonia has disappeared, a few drops of ferric chloride are added and the presence of benzoic acid is indicated by the formation of a flesh-coloured precipitate. If any ammonia is left in the solution, a reddish-brown precipitate of ferric hydrate is obtained, so that it is essential that all traces of this disturbing substance are removed before applying the final test.

**Salicylic Acid.** This is detected in exactly the same manner as is described above for the detection of benzoic acid. On addi

tion of ferric chloride, a solution of salicylic acid produces a characteristic violet colour, the intensity of which is somewhat proportional to the amount of salicylic acid present.

**Hydrogen Peroxide.** As hydrogen peroxide decomposes into free oxygen and water soon after its addition to milk, it is impossible to detect this substance by means of the usual reagents. The oxygen liberated, however, considerably modifies the enzymes present, and it is upon this fact that several inferential tests for detecting hydrogen peroxide are based. The immediate reductase reaction (see p. 89) is destroyed by hydrogen peroxide, and the catalase (see p. 91) destroyed in proportion to the amount added.

Before the hydrogen peroxide has decomposed it may be detected by the peroxidase reaction (see p. 91).

**Hypochlorites.** Although hypochlorites have been suggested as milk preservatives they have not been extensively used as the amount required to produce any appreciable effect also adversely affects the taste and odour. Milk containing hypochlorites does not give the usual starch-iodide reaction even with as large a quantity as 50 parts of available chlorine per 100,000.

**Detection of Added Colouring Matter.** The following are the provisionally official methods of the American Association of Official Agricultural Chemists.

Warm about 150 c.cms. of milk in a basin over a flame and add about 5 c.cms. of acetic acid, after which slowly continue the heating almost to the boiling point whilst stirring. Gather the curd, when possible, into one mass by means of the stirring rod, and pour off the whey. If the curd breaks up into small flecks, separate from the whey by straining through a sieve or muslin. Press the curd free from adhering liquid, transfer to a small flask, and macerate for several hours (preferably overnight) in about 50 c.cms. of ether, the flask being tightly corked and shaken at intervals. The ether is finally decanted from the curd and is examined for annatto, the curd being reserved for the detection of aniline orange and caramel.

*Annatto.* After evaporation of the ether, the fatty residue is made alkaline with caustic soda and, whilst still warm, poured upon a very small wet filter paper. After the solution has passed through, wash the fat from the paper with a stream of water and dry the paper. If, after drying, the paper is coloured orange, the presence of annatto is indicated. This may be confirmed by adding a drop of stannous chloride solution, which, in the presence of annatto, produces a characteristic pink on the orange-coloured paper.

*Aniline Orange.* The curd of an uncoloured milk is perfectly white after complete extraction with ether, as is also that of a milk coloured with annatto. If the extracted curd is distinctly dyed an orange or yellowish colour, the presence of aniline orange is indicated. To confirm this, treat a lump of the fat-free curd with a little strong hydrochloric acid. If the curd turns pink, the presence of aniline orange is assured.

Aniline orange may also be detected by Lythgoe's method which consists of the addition of 10 c.cms. of concentrated hydrochloric acid to an equal volume of milk in a porcelain dish and imparting a rotary motion to the contents. If any appreciable amount of aniline orange is present, a pink colour is at once imparted to the curd particles as they separate.

*Caramel.* If the fat-free curd is coloured a dull brown, caramel is suspected. Shake a lump of the curd with concentrated hydrochloric acid in a test tube and heat gently. In the presence of caramel the acid solution will gradually turn a deep blue, as will also the white fat-free curd of an uncoloured milk, while the curd itself does not change colour. It is only when this blue colouration of the acid occurs in conjunction with a brown-coloured curd, which itself does not change colour, that caramel can be suspected, as distinguished from the pink colouration produced by aniline orange under similar circumstances.

## Analysis of Milk Products

**Cream.** The normal constituents can be determined by employing the usual methods of milk analysis after suitable detection with water (*vide* p. 66). The amount of cream used for dilution, however, should be weighed and not measured volumetrically. The total solids should be determined by evaporation, and Richmond recommends the addition of an equal volume of alcohol to accelerate drying. Richmond also finds that the total solids and fat bear the relation expressed by the formula:

$$\text{Fat} = 1.102\ \text{Total Solids} - 10.2$$

Thickening agents are sometimes added to cream for the purpose of increasing the viscosity and thus produce the appearance of a cream of high fat content. The usual agents employed are gelatine, starch, and saccharate of lime (viscogen).

Small quantities of gelatine may be detected by Stokes' method.[6] Mercury is dissolved in twice its weight of concentrated nitric acid (1.42) and the solution diluted with twenty-five times its volume of water. To 10 c.cms. of cream add an equal bulk of mercuric nitrate solution and about 20 c.cms. of cold water. Shake vigourously and filter after standing for a few minutes. Inability to obtain a clear filtrate indicates the presence of gelatine and this may be confirmed by adding an equal volume of a saturated solution of picric acid. A yellow precipitate is produced by gelatine in a cold solution.

Starch is detected by the formation of a blue colouration on addition of a solution of iodine in potassium iodide.

Saccharate of lime may be detected by the estimation of either the lime in the ash or by the lactose determination. The lime in normal samples averages about 22.4 per cent of the ash and any perceptible increase over this amount is suspicious. Similarly an abnormally high polarimeter reading, equivalent, when calculated as lactose, to more than 52.5 per cent of the solids not fat, should also be regarded with suspicion.

**Skim Milk.** The usual methods of milk analysis may be applied.

**Condensed Milk.** About 30 grms. of milk are weighed out and, after boiling with 50 c.cms. of water, the solution is cooled and made up to 100 c.cms. The methods of analysis described above under milk may then be applied, but longer extraction should be given if the Adams process is used for the estimation of the fat.

In sweetened samples the cane sugar is determined by subtracting the sum of the fat, lactose, proteids, and ash, from the total solids.

## Enzymes

Although the presence of enzymes in milk has been an established fact for many years, it is only comparatively recently that the origin of these ferments has been seriously considered. The nature and characteristics of these bodies suggests that they are derived from the blood and the results of various experimenters show that they are largely associated with the cells invariably found in milk samples. Whilst the greater portion of the enzyme activity of milk is anchored to the cells and may, consequently, be removed by filtration, there is also present a smaller quantity of extra cellular activity. This is not surprising when the rapid metabolic changes taking place during the secretion of milk are considered. Certain enzymes, such as Schardinger's reductase, occur in amounts which vary directly with the fat content and, unless, this enzyme is almost entirely extra cellular, the cells should also vary somewhat with the fat content. Although various hypotheses have been advanced as to the effect of enzymes in milk, the author believes that too much importance has been attached to the qualitative and too little to the quantitative tests for these substances. The amylase content of normal milk is equivalent to about 0.4 grm. of starch per 100 c.cms. of milk per hour. The catalase in 100 c.cms. liberates from hydrogen peroxide 10 c.cms. or 0.014 grm. of oxygen in two hours, whilst Babcock and Russell's

figures show the galactase activity to be capable of digesting approximately 1 per cent of proteids in milk in twenty-four hours. Compared with the activity of the normal secretions of the alimentary tract, these quantities are so small as to possess but little, if any, physiological significance. Pathological conditions such as mastitis, which involve inflammatory processes of the udder, increase the cell content and, consequently, also the enzyme activity of milk, whilst heating of the milk to temperatures of 75° C. and over, weaken and finally destroy the enzymes. As an aid to the diagnosis of such conditions and for the control of pasteurisation, the determination of the ferment activity may be found desirable and for this purpose the following methods have been proved to be satisfactory. The determinations that can be most conveniently carried out in routine work and which do not require special apparatus are the reductase and peroxide tests: the catalase and amylase follow next in order of facility whilst the others are of more scientific interest than practical utility.

**Reductase.** To 10 c.cms. of milk, add 1 c.cm. of Schardinger's reagent (100 parts water and 5 parts each of formalin and a saturated alcoholic solution of methylene blue) and heat to 43°–45° C.: the time required for decolourisation is noted. The reoxidation of the surface layers by the air may be entirely prevented by adding a small quantity of paraffin, but the cream layer usually gives the necessary protection.

Any desired temperature, not exceeding 60° C., may be used for carrying out this test, but whatever temperature is chosen must be adhered to in order that the results may be strictly comparative. In most laboratories, a temperature of 43°–45 C. will be found convenient as the water bath employed for liquid agar media is usually maintained at this temperature.

This ferment is not present in every sample of milk from individual cows, being frequently absent from animals whose offspring are still suckling and in animals whose lactation period is just commencing (Schern) but the author has invariably found it to be present in mixed market samples. Romer and

Sames have found that it does not decolourise, or only completely so, in the fore milk and that the time required for decolourisation becomes less as the milking proceeds. This corresponds to the relative frequency of the fat content and on this connection the following figures calculated from some of the author's results are of interest:

TABLE XXXVI

RELATION OF BUTTER FAT TO REDUCTASE CONTENT

| Butter Fat Content. | Average Time for Reduction. |
|---|---|
| | Minutes. |
| Less than 3.4 | 15 |
| 3.4 to 3.6 | 17 |
| 3.7 to 3.9 | 16 |
| 4.0 to 4.2 | 14 |
| 4.3 to 4.5 | 13 |
| 4.6 to 4.8 | 10 |
| More than 4.9 | 7 |

The following results of the author show that there is no relation between the bacterial content of milk and the reductase test or hastened reductase test as it is sometimes known as (cf. p. 24):

TABLE XXXVII

RELATION OF BACTERIAL COUNT TO REDUCTASE CONTENT

| Bacterial Count per C.cm. Agar 48 Hrs. at 37° C. | Average Terms of Reduction. Minutes. |
|---|---|
| Less than 10,000 | 13 |
| 10,001 to 50,000 | 16 |
| 50,001 to 100,000 | 14 |
| 100,001 to 200,000 | 17 |
| 200,001 to 300,000 | 17 |
| 300,001 to 400,000 | 19 |

**Peroxidases.** The detection of this ferment may be carried out by any of the following methods, all of which are reliable.

*Rothenfusser's Method.* Two solutions are required: (1) a 6 per cent solution of pure para phenylenediamine hydrochloride, and (2) a 1.8 per cent solution of crystallised guiacol in 96 per cent alcohol. 15 c.cms. of No. 1 are added to 135 c.cms. of No. 2 and the mixture preserved in an amber-coloured bottle. To 10 c.cms. of milk add 0.5 c.cm. of the reagent and 3 drops of hydrogen peroxide (3 per cent). A blue violet colouration indicates a positive peroxidase reaction.

*Wilkinson and Peter's Method.* To 10 c.cms. of milk add 1 c.cm. of a 10 per cent solution of benzidine in 96 per cent alcohol, 3 drops of 30 per cent acetic acid and finally 2 c.cms. of 3 per cent hydrogen peroxide. Peroxidases produce a blue colouration which is usually localised in the precipitated caseinogen.

*Bellei's Method.* To 10 c.cms. of milk, add three drops of a 1.5 per cent aqueous solution of ortol and two drops of a 3 per cent hydrogen peroxide solution. A red colouration indicates the presence of peroxidases.

Peroxidases, like reductase, are more concentrated in the cream layer of milk though it is impossible to establish any definite parallelism between the butter fat content and the density of the peroxidase reaction.

**Catalase.** The activity of this ferment is estimated by mixing 15 c.cms. of milk and 5 c.cms. of 2 per cent hydrogen peroxide in a special tube devised for this purpose by Lobeck. In this apparatus the oxygen liberated is collected and measured in a graduated tube previously filled with water. The liberation of the oxygen is accelerated by incubation at blood heat for two hours. Fresh milk usually evolves one to three cubic centimetres of oxygen and results materially higher than these are usually indicative either of excessive bacterial contamination or of excessive amounts of cellular elements produced by physiological or pathological irritations of the udder.

**Amylase.** Into each of 10 test tubes, 10 c.cms. of milk are

placed and to these are added 0.1, 0.2, 0.3 up to 1 c.cm. of a 1 per cent solution of soluble starch prepared by boiling with distilled water and cooling. After shaking, the tubes are placed in a bath at 43°–45° C. for one hour and then rapidly cooled. To each is added 1 c.cm. of a solution of iodine in potassium iodide (1 grm. iodine, and 2 grms. potassium iodide in 300 c.cms. of water), and the colour noted immediately after shaking. The recording of the tints admits of no delay, as the colours rapidly fade and all the tubes may regain their original shades. A yellow tint indicates total conversion of the starch to sugar, and a blue one unchanged starch: the correct reading is where the yellow just commences to take on a greyish tint. With normal fresh milk this will usually be found between the third and fifth tubes. The indications of this test are similar to those of the catalase test, both being based on the quantity of cellular elements.

**Galactase.** The Babcock and Russell method is probably the most reliable for the estimation of this ferment, but the time required for its execution is so long that it is never carried out in routine examinations. The milk is incubated at blood heat for 53 days with the addition of sufficient thymol to prevent bacterial development and an estimation of the soluble nitrogen then made. The difference between this result and that originally present indicates the amount produced by the enzyme activity. This is usually less than 1 per cent per day.

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## CHAPTER IV

### BACTERIA IN MILK

Milk, like other secretions, is sterile at the moment of secretion but it is usually impossible to obtain it from the udders of cows in this condition even though every precaution be taken and all operations are conducted under strictly aseptic conditions. Many have held that bacteria may be transferred to milk directly from the blood stream of healthy cows, but this view is now generally regarded as erroneous.

Amongst the earliest investigators to doubt the sterility of the udder were Balley and Hall [1] who concluded from their experiments that the milk cistern might be the seat of bacterial development and one source of bacterial contamination of milk. Ward [2] carefully examined the udders of 19 milch cows from 5 dairies and found that although the animals were tubercular, the udders were normal. He found that all the lactiferous ducts of the cows were contaminated throughout with bacteria of which the majority were cocci. From his studies on the anatomy of the udder Ward concluded that with the possible exception of the sphincter muscle, at the lower end of the teat, no obstruction capable of excluding bacteria from the milk cistern exists. This would indicate that the source of contamination of milk even in the udder is external and that the portal of entry is the teat.

Henderson [3] examined a number of cultures from seven normal udders and obtained growth in 76 per cent, but two cases of unexpanded udders from heifers gave sterile cultures from the milk cistern, ducts, and parenchyma.

The intra-mammary contamination of milk in healthy udders is usually small, and, although in some exceptional cases counts

as high as 15,000 per c.cm. have been obtained, it is probable that at least a portion of this number was due to external contamination caused by faulty aseptic conditions of milk withdrawal.

Sedgwick and Batchelder [4] found that with moderate precautions on the part of the milker, the organisms in fresh milk may not exceed 500 to 1000 per c.cm., but if ordinary flaring pails were used with more or less disturbance of the bedding and shaking of the udder, the count may be 30,000 or even more.

Park [5] found the average count from six separate cows, five hours after collection, to be 4000 per c.cm. (minimum 400 per c.cm.) and the average of 25 cows as 4550.

McConkey [6] observed that, with ordinary care and cleanliness, it was possible to obtain milk containing less than 1500 bacteria per c.cm. and that such milk should not contain gas formers in less than 50 c.cms.

Von Freudenreich [7] thought it would be easy to obtain sterile milk by using strict asepsis but soon found otherwise. Such milk invariably contained 250–300 bacteria per c.cm. though the hands of the milkers and the teats of the cows were washed with soft soap and sterile water, then with servatol soap and sterile water, and, finally with sterile water and then dried on a sterile towel. The milkers' hands were smeared with lanoline and the fore milk rejected. The bacterial content of the mixed milk of 28 cows so milked varied from 65–680 per c.cm. Von Freudenreich and Thoni [8] from a further series of experiments concluded that freshly drawn milk, even when every precaution is taken against contamination, always contains bacteria; they found that these were mostly cocci and were derived from the udder. A summary of the more important attempts to obtain sterile milk is as follows:

| | |
|---|---|
| Von Freudenreich, | 200–300 per c.cm. |
| Szasz, | 2 samples sterile. Average of 11 = 2700 per c.cm. |
| Hesse, | 1600 per c.cm. |
| Marshall, | 295 per c.cm. |
| Lux, | 0 to 6800 per c.cm. |

| | |
|---|---|
| Kolle, | 80 to 15,000 per c.cm.<br>33 per cent less than 300.<br>50 per cent less than 500.<br>4.7 per cent 700–800 |
| Willem and Minne, | 1 to 5 per c.cm. |
| Willem and Miele, | 0 to 37 and 4 to 218 per c.cm. |
| Siebald, | (1) Without protective measures. Under 10 to several thousands.<br>(2) After soaping the udder. 0 to 85 per c.cm.<br>(3) After soaping the udder and disinfecting with alcohol and milking through sterile tubes, 0 to 12 per c.cm. |

All these numerous experiments prove conclusively that some intra-mammary contamination of milk exists and it will be advisable next to consider the nature of this.

Like Ward [2] Freudenreich [9] found that udder contamination in healthy cows was mostly caused by cocci, but Str. lacticus (Heinemann) was only found in three cases out of a total of fifteen. B. coli was never found. The organisms found by Henderson [3] were streptococci, staphylococci and pseudo diphtheriæ and similar results were obtained by Bergey.[10]

From these and other results it would appear that cocci, some of a proteolytic nature, form the prevailing type found in udders and that the lactic acid producing bacteria, both coliform and Str. lacticus, are usually absent. Some of the streptococci and staphylococci found in milk produced under strictly aseptic conditions are biochemically similar to those usually associated with inflammatory processes but are commonly of much lower virulence.

Experiments on the viability of various organisms in the environment of milk ducts has shown that they rapidly die, many bacteria disappearing within a few days. Savage [11] inoculated the teats of goats with streptococci of both bovine and human origin and found that the infecting organism usually died in a few weeks, although in one case the streptococci persisted for over seven months. The streptococci from human sources were usually less viable.

Although the majority of the evidence available favours the hypothesis that the source of intramammary contamination is external it is difficult to establish this entirely on account of the impossibility of putting the ducts and cisterns in a sterile condition. Once infection of the udder has occurred, the organism, finding the mammary secretion an excellent pabulum for development, persists and the small quantity of milk remaining from one milking contaminates the next, the process being repeated until the cow becomes dry. That the amount of milk allowed to remain in the udder has a very material influence upon the bacterial count of the milk obtained at the next milking is shown by the experiments of Stocking,[12] who found as the average of ten experiments 6542 bacteria per c.cm. in milk obtained after thoroughly stripping the udder as against 11,324 per c.cm. when this was neglected. The importance of this factor is now well recognised in large dairies using milking machines, for it is invariably the custom to take out the last strippings by hand, owing to the impossibility of obtaining this milk by means of the machine. This hand-milked secretion often contains more bacteria than the portion immediately preceding it, due, Stocking suggests, to more vigorous manipulation of the udder dislodging bacteria from the ducts and which remained there during the earlier part of the milking. The contaminated milk left in the ducts is, of course, mostly discharged in the fore milk and a decreasing count is obtained as milking proceeds. Stocking [12] reports the following results in this connection as the averages of four experiments:

| | Bacteria per c.cm. |
|---|---|
| Streams 1 and 2 | 10,143 |
| Streams 5 and 6 | 2,347 |
| Streams 9 and 10 | 272 |
| Streams 13 and 14 | 382 |
| Strippings | 204 |

The influence of the rejection of the contaminated fore milk was shown by the following figures:

| | Bacteria per c.cm. | | |
|---|---|---|---|
| | Total. | Acid. | Liquefying. |
| Fore milk rejected..... | 499 | 99 | 33 |
| Fore milk retained..... | 522 | 189 | 9 |

Backhaus [13] reports 10,400 bacteria per c.cm. in fore milk as against practically sterile strippings whilst the author in one instance obtained 50,000 per c.cm. in the fore milk, 4000 in the middle milk and 500 in the strippings. The advantage obtained by the rejection of the fore milk is usually much greater than is indicated by Stocking's results reported above, but this factor is largely determined by the precautions observed in other directions and may be but a minor one if the udders are thoroughly stripped and kept clean between and during milking operations. This so-called intramammary contamination, which is really external contamination, though conveyed to the milk whilst in the udder, is, however, only a fraction of the external contamination that reaches the milk directly; this is especially true of ordinary market milk. The external contamination increases at every stage between milking and delivery to the consumer and is very diverse in character. The chief sources of contamination are:

(1) During milking. Bacteria from dirty udders, flanks, and hands of milkers: also aerial contamination with dust of food or litter.

(2) During handling. Dirty containers, strainers and cooling apparatus.

The influence of bodily cleanliness of the cow on the bacterial count of the milk obtained has been investigated on several occasions. Backhaus [13] found 20,600 bacteria per c.cm. in the milk of brushed cows as against 170,000 per c.cm. from unbrushed cows. Stocking [12] reports the following results:

| | Bacteria per c.cm. | | |
|---|---|---|---|
| | Total. | Acid. | Liquefying. |
| Brushed | 2268 | 381 | 117 |
| Unbrushed | 1207 | 213 | 59 |

Wiping the udders with a damp cloth previous to milking reduced the bacterial count from 7,058 to 716 per c.cm. Similar results are also reported by Harrison.[14] Orr [15] exposed plates of nutrient medium for two minutes during milking and afterwards incubated them for four days at 20° C. The results are given in Table XXXVIII.

Table XXXVIII

| Housing of the Cows. | Conditions of the Cows. | No. of Experiments. | Average Count per Plate. |
|---|---|---|---|
| Summer, all cows out | Untouched | 7 | 440 |
| Summer, all cows out | Udders and flanks washed and brushed | 3 | 170 |
| Winter, cows indoors | Untouched | 3 | 4752 |
| Winter, cows indoors | Udders and flanks brushed but not washed | 3 | 1752 |
| Winter, cows indoors | Udders and flanks brushed and washed and left moist | 6 | 230 |
| Winter, cows indoors | Udders and flanks brushed, washed and dried | 3 | 444 |

The practice of moistening the hands of the milkers by the first milk streams was shown by Backhaus to increase the bacterial count from 5600 to 9000 per c.cm. The effect of the character of the litter and the food employed is very marked as is also that of the influence of time of feeding. The tendency of the litter to dust formation is a factor in this direction.

TABLE XXXIX

BACTERIA IN LITTER (BACKHAUS)

| Litter. | Organisms, Per Gram. |
|---|---|
| Peat | 2,000,000 |
| Good straw | 7,500,000 |
| Bad straw | 10,000,000 |

The milk obtained contained

| | Bacteria per C.cm. |
|---|---|
| With peat litter | 3500 |
| With straw litter | 7330 |

Backhaus also found that oil cake averaged 450,000 bacteria per gram and bran 1,362,000 per gram, and there is no doubt that other dry foods also contain similar large numbers of organisms. Moist foods such as ensilage would have no effect if entirely consumed but would be equally objectionable as other foods if allowed to dry.

Stocking [12] reports the following results in connection with experiments on the influence of feeding before and after milking.

HAY AND CORN

| | Total. | Acid. | Liquefying. |
|---|---|---|---|
| Given after milking | 2096 | 790 | 108 |
| Given before milking | 3506 | 1320 | 196 |

DRY CORN

| | Total. | Acid. | Liquefying. |
|---|---|---|---|
| Given after milking | 1233 | 297 | 118 |
| Given before milking | 3656 | 692 | 123 |

The results of Harrison [14] are equally interesting. The organisms falling on an area equal to a circle having a diameter of 12 inches were found to vary from 12,210 to 42,750 during bedding, feeding and cleaning up, whilst one hour later similar tests gave only 483 to 2370 organisms.

Orr [15] by exposing plates of nutrient medium for five minutes and afterwards incubating for four days at 20° C. obtained from 1260 to 4500 organisms per 113 square inches (area of circle 12 inches in diameter). The author has found that in clean, well-ventilated cow byres as low a germ content as 200 per 113 square inches could be attained when tested with plates of nutrient agar for five minutes and incubated at 37° C. for forty-eight hours. Coliform bacilli, as shown by neutral red lactose agar plates, were usually absent.

The influence of milk containers is also well marked. Backhaus found that fresh milk which originally contained only 6600 bacteria per c.cm. was increased in germ content to 97,000 per c.cm. by passage through six containers. Wooden pails were the most objectionable in this respect as they averaged 280,000 germs as against 1690 for galvanized iron and 1105 for enamelled ware. Pails after rinsing contained 28,600 organisms and sterilized pails only 1300. Harrison [14] also investigated the cleansing of cans; by rinsing the vessels with 100 c.cms. of sterile water he obtained the following results:

| | Bacteria per c.cm. |
|---|---|
| Improperly cleaned cans | 215,000–806,320 |
| Washed with tepid water and scalding | 13,080– 93,400 |
| Washed with tepid water and steaming 5 mins. | 355– 1,792 |

Cloth and absorbent cotton strainers may also be a source of bacterial contamination unless proper precautions are taken.

Milk coolers of the open type may introduce contamination from both the cooler itself and from the air. This is well exemplified by the results both of Orr [15] and the author. (Table XL.)

Two other sources of milk contamination are water and cow fæces. It is obvious that all the water used for cleansing and

Table XL

EFFECT OF MILK COOLERS

Average of Four Experiments (Orr)

| | Bacteria per c.cm. in Milk. | |
|---|---|---|
| | Agar 48 Hrs. at 37° C. | Gelatine 96 Hrs. at 20° C. |
| Before cooling | 26,000 | 39,000 |
| After cooling | 48,000 | 104,000 |
| Author's results: | | Coliform. |
| Before cooling | 25,000 | 4 |
| After cooling | 400,000 | 3,500 |
| After thorough cleansing of coolers: | | |
| Before cooling | 28,000 | 2 |
| After cooling | 30,000 | 8 |

rinsing the various utensils that come in contact with the milk at various stages cannot all be sterilised, so that milk will contain a number of the bacteria usually found in water supplies.

Cow fæces may also be conveyed to milk by falling into milking pails after becoming dried upon the udders and flanks of the cows. This danger may be eliminated as has previously been pointed out by washing these portions of the beasts. Savage [16] gives several analyses of fresh cow excreta. (Table XLI.)

From this general consideration of the various sources of milk contamination it is obvious that milk even whilst fresh may contain large numbers of an almost infinite variety of organisms. Before taking up the methods of examination for these organisms it will be advisable to consider the effect of storage, for milk samples are rarely taken of the product in a fresh condition. This point is also important in considering the conditions requisite for preventing bacterial multiplication

TABLE XLI

BACTERIA IN COW FÆCES (SAVAGE)

| Source. | Organisms per Gram. | | |
|---|---|---|---|
| | B. coli. | Streptococci. | B. enteritiditis sporogenes Spores. |
| Cow No. 1 | 100,000– 1,000,000 | 10,000– 100,000 | 100–1000 |
| 2 | 1,000– 10,000 | 100,000–1,000,000 | 10– 100 |
| 3 | 1,000,000–10,000,000 | More than 10,000,000 | 10– 100 |
| 4 | 1,000,000–10,000,000 | 100,000–1,000,000 | 100–1000 |

in the interval that elapses between sampling and the laboratory examination.

Park [17] took two samples of milk, one containing 3000 organisms per c.cm. (agar forty-eight hours at 37° C.) and the other 30,000 per c.cm. and stored portions at various temperatures. After various intervals of time the bacterial counts were again taken with the results shown in Table XLII.

The author has made similar tests but, in addition to the total bacterial count, an estimation was made of the B. coli group by plating on rebipelagar (neutral red bile salt agar) and incubating at 37° C. for twenty-four hours. The total bacteria were counted on +1.0 per cent nutrient agar after forty-eight hours incubation at 37° C.

It will be noticed in both these series of experiments, and especially in Park's, that at the lower temperature there is at first an apparent diminution in the total bacterial count and that this phenomenon is more definite and more prolonged at the lowest temperature used. These observations have been confirmed by many experimenters and led to the hypothesis that milk possessed a weak, though definite bactericidal action: this is usually referred to as the germicidal action of milk. M. J. Rosenau [18] thoroughly investigated this phenomenon and concluded that no true germicidal action took place, but that

## Table XLII

Upper figures represent sample No. 1. Original count 3,000.
Lower " " " No. 2. " " 30,000.

| Temperatures, ° F. | Time which Elapsed before Making Test. | | | |
|---|---|---|---|---|
| | 24 Hours. | 48 Hours. | 96 Hours. | 168 Hours. |
| 32 | 2,400<br>30,000 | 2,100<br>27,000 | 1,850<br>24,000 | 1,400<br>19,900 |
| 39 | 2,500<br>38,000 | 3,600<br>56,000 | 218,000<br>4,300,000 | 4,200,000<br>38,000,000 |
| 42 | 2,600<br>43,000 | 3,500<br>210,000 | 500,000<br>5,760,000 | |
| 46 | 3,100<br>42,000 | 12,000<br>360,000 | | |
| 50 | 11,600<br>89,000 | 540,000<br>1,940,000 | | |
| 55 | 18,800<br>187,000 | 3,400,000<br>38,000,000 | | |
| 60 | 180,000<br>900,000 | 28,000,000<br>168,000,000 | | |
| 68 | 450,000<br>4,000,000 | 25,000,000,000<br>25,000,000,000 | | |
| 86 | 1,400,000,000<br>14,000,000,000 | | | |
| 94 | 25,000,000,000<br>25,000,000,000 | | | |

fresh milk appeared to act as a weak antiseptic. Vigorous shaking of the samples demonstrated that the reduction in count was more apparent than real and suggested that the

## Table XLIII

### Author's Results

**Agar 48 hrs. at 37° C. and Rebipelagar 24 hrs. at 37° C.**
Original milk = 6700 total bacteria. 3 B. coli per c.cm.

| Temp. ° F. | Organisms. | Time which Elapsed before Making Test. | | | | | |
|---|---|---|---|---|---|---|---|
| | | 6 Hours. | 24 Hours. | 48 Hours. | 72 Hours. | 96 Hours. | 120 Hours. |
| | Total bacteria.... | 6300 | 16,000 | 14,000 | 23,000 | 33,600 | 57,000 |
| 38 | B. coli..... | 3 | 1 | 0 | 0 | 0 | |
| | Total bacteria.... | 6500 | 20,800 | 27,200 | 34,000 | 87,000 | 108,000 |
| 43 | B. coli..... | 5 | 3 | 0 | 1 | 3 | |
| | Total bacteria.... | 7800 | 150,000 | 520,000 | 2,960,000 | 39,200,000 | 210,000,000* |
| 51 | B. coli..... | 4 | 22 | 760 | 1,980,000 | 7,200,000 | 21,000,000 |
| | Total bacteria.... | ..... | 208,000 | 84,000,000 | 288,000,000* | | |
| 59 | B. coli..... | ..... | 2,570 | 3,460,000 | 27,000,000 | | |
| | Total bacteria.... | ..... | 720,000 | 300,000,000* | | | |
| 64 | B. coli..... | ..... | 19,200 | 110,000,000 | | | |
| | Total bacteria.... | ..... | 8,800,000 | 320,000,000* | | | |
| 70 | B. coli..... | ..... | 1,900,000 | 900,000 | | | |

* Curdled.

organisms had aggregated into clusters under the influence of agglutinins. The so-called germicidal action was also found to be specific but the specificity of different samples was variable. Further proof of the fact that this phenomenon must be attributed to agglutinins rather than to bacteriolysins was found in the behaviour of heated milk. Heating to 56° C. for thirty minutes, a condition which destroys bacteriolysins, weakens but does not entirely inhibit the action; it is entirely destroyed at 75° C.

St. John and Pennington [19] found that milk, after heating to 79° C. for twenty minutes, not only failed to show an apparent diminution in the number of organisms but also showed a much greater rate of bacterial development throughout the period of observation. They point out that this is a serious objection to pasteurisation as a reinfected heated product exerts no restraining effect upon the invading organisms and may, therefore, be more infective than raw milk receiving the same original contamination.

Stocking,[20] who investigated this question, concluded that the apparent diminution of organisms capable of development on solid media was really due to bacteria finding the milk a pabulum to which they are unaccustomed and consequently died at a faster rate than they could multiply; he found that this resting stage was scarcely observable with common lactic acid organisms which appeared to develop more or less rapidly and continuously from the moment of their introduction into the milk. The absence of a " germicidal effect " with common lactic acid organisms was confirmed by Rosenau and others and supports rather than impairs the validity of the agglutination hypothesis by accentuating its specificity. The resting stage pointed out by Stocking must also be a factor, but cannot wholly account for it as it fails to explain the comparative absence of the phenomenon in heated milk unless it is assumed that heating has resulted in chemical changes that have produced a more favourable environment for bacterial development. Once this resting period or germicidal phase has passed,

bacterial development sets in, the rapidity of which depends upon the temperature at which the sample is stored. The organisms that have gained admittance to the milk do not all find that substance a suitable medium for reproduction, but certain classes develop rapidly and ultimately one or more of these classes predominates. The bacteria that reproduce most rapidly may be roughly divided into three groups according to their biochemical characteristics, viz., acid producers, proteolytic, and inert organisms. Ayers and Johnson [21] made a fourth general division by separating the alkali producers, but this group is usually included in the inert group. The classification was based upon the behaviour of the organisms on litmus lactose gelatine, the acid producers being those capable of producing red colonies, the proteolytic being liquefiers, and the balance, having no well-defined characteristics on this medium, the inert group. The acid producers may be subdivided into two further groups according to their ability to ferment lactose with the production of gas. This separates the coliform organisms, which produce hydrogen and carbon dioxide from lactose in addition to lactic acid, and the ordinary lactic acid organisms which do not give any gaseous products.

Although different samples of milk will all show varying rates of development of the various groups, a general discussion of this point will, perhaps, be facilitated by consideration of a concrete example. Table XLIV shows the results of a daily examination of a sample of milk kept comparatively cool.

All three groups, in this example, developed rapidly, the greatest relative increase being shown by the coliform organisms, until a maximum was reached at the end of five days. At this stage the acidity was 44° and this amount was evidently sufficient either alone or in conjunction with the other products of metabolism, to restrain the rate of production. The coliform organisms were the first to be affected, although the other acid producers and to an even smaller degree, the liquefiers, were restrained. On the tenth day the liquefiers commenced

## TABLE XLIV

(AUTHOR)

| Temperature Fahrenheit. | Day. | Coliform Organisms. | Liquifiers. | Acid Producers. | Inert. | Total. | Acidity Degrees |
|---|---|---|---|---|---|---|---|
| 56 | 1 | 1 | 800 | 28,000 | 23,200 | 53,000 | 15 |
| 58 | 2 | 1,000 | 750,000 | 3,000,000 | 1,250,000 | 5,000,000 | 15 |
| 50 | 3 | 400,000 | 4,000,000 | 15,000,000 | 14,000,000 | 33,000,000 | 16 |
| 50 | 4 | 1,600,000 | 15,000,000 | 68,000,000 | 26,000,000 | 109,000,000 | 22 |
| 50 | 5 | 4,500,000 | 25,000,000 | 900,000,000 | 575,000,000 | 1,300,000,000 | 44 |
| 48 | 6 | 2,800,000 | Plates Liquified. Sample Curdled. | | | | 68 |
| 48 | 7 | 1,900,000 | 40,000,000 | 800,000,000 | 460,000,000 | 1,300,000,000 | |
| 50 | 8 | 600,000 | 50,000,000 | 750,000,000 | 180,000,000 | 980,000,000 | 83 |
| 48 | 9 | 200,000 | 60,000,000 | 1,200,000,000 | 240,000,000 | 1,500,000,000 | |
| 48 | 10 | 48,000 | 20,000,000 | 400,000,000 | 130,000,000 | 550,000,000 | 91 |
| 48 | 11 | 18,000 | 10,000,000 | 340,000,000 | 50,000,000 | 400,000,000 | |
| 48 | 12 | 4,500 | 4,100,000 | 500,000,000 | 45,900,000 | 550,000,000 | 93 |
| 48 | 13 | 890 | 1,000,000 | 700,000,000 | 99,000,000 | 800,000,000 | |
| 48 | 14 | 440 | <1,000,000 | 670,000,000 | 80,000,000 | 750,000,000 | |
| 50 | 15 | 100 | <1,000,000 | 750,000,000 | 50,000,000 | 900,000,000 | 98 |

to gradually decrease and a few days later it was impossible to make an accurate estimation of their number owing to the overgrowth of acid producers. The inert group developed well during the first period and, after a reduction at the tenth day period, persisted to the end of the experiment. The sample ultimately developed a prolific growth of torulæ.

In considering the relative development of various groups in milk, due regard must always be given to the two important factors, viz., temperature and initial content, that determine the results.

The effect of temperature was carefully investigated by Conn and Esten,[22] who plated out practically fresh milk usually containing 20,000 bacteria per c.cm. on litmus lactose agar and found that they were able to distinguish no less than 15 different groups merely by their macroscopic appearance. They made two series of experiments, the first at 37° C., 20° C., and 10° C. and the second at 20° C., 10° C., and 1° C. The plating intervals were:

37° C. at 2 hour intervals
20° C. at 6 hour intervals
10° C. at 12 hour intervals
1° C. at 1 day intervals.

The main conclusions, as summarised by Conn and Esten, were:

(1) The effect of variations of temperature upon the development of different species of bacteria in milk is not always the same under apparently identical conditions. In spite of such variations, there seems to be clearly discernible a normal development of bacteria associated with different temperatures.

(2) There is, in all cases, a certain period at the beginning when there is no increase in the total number of bacteria. During this period some species are multiplying whilst others are apparently dying. The length of this period depends upon the temperature. At 37° C. it is very short, while at 10° C. it may last from six to eight days, since, at this temperature,

milk may, in six days, actually contain fewer bacteria than when fresh.

(3) After this preliminary period, there always follows a multiplication of bacteria; but the types that develop differ so markedly, that samples of the same milk kept at different temperatures are, at later periods, very different in their bacterial content, even though they contain the same number of bacteria.

(4) The development of the ordinary lactic species Bact. lactis acidi (Str. lacticus), in practically all cases checks the growth of other species of bacteria, and, finally, kills them, since the bacteria regularly decrease in actual numbers after the lactic bacteria have become very abundant.

(5) In practically all samples of milk kept at 20° C., the multiplication of the Str. lacticus * begins quickly and progresses with great rapidity. They grow so rapidly that they produce acid enough to curdle the milk in about forty hours, the growth of other species being held in check. Milk when curdled at this temperature shows a smooth acid curd, with no gas bubbles.

(6) A totally different result appears in milk kept at 37° C. The results are somewhat more variable than at 20° C. Occasionally the Str. lacticus grows vigorously at this temperature, but the common result is a development of the B. lactis ærogenes type. It forms a curd full of gas bubbles. If B. coli communis is in the milk, this also grows luxuriantly at 37° C.

(7) In milk kept at 10° C., neither of the types of bacteria seems to be favoured. The delay in growth lasts two to three days, after which all types of bacteria appear to develop somewhat uniformly. Sometimes the lactic bacteria develop abundantly, sometimes only slightly. The neutral bacteria always grow rapidly, and the liquefiers in many cases become abundant. In time, the milk is apt to curdle, commonly with

* Str. lacticus has been substituted for B. lactis acidi (Hueppe) in order to avoid confusion with B. acidi lactici (Escherich).

an acid reaction, but it never shows the predominance of Str. lacticus found at 20° C.

(8) From our experiments there seems to be no difference between the effect of 10° and 1° upon the bacteria, except upon the rapidity of growth. 1° C. very markedly checks the growth of bacteria; but, later they grow in large numbers. As at 10° C., the lactic bacteria fail to outgrow the other species, so that all types develop abundantly. A few species appear to be particularly well adapted to this low temperature and are especially abundant at the end of the experiment.

(9) The curdling point appears to be quite independent of the number of bacteria present. In one sample at 37° C., the milk curdled with only 8,000,000 organisms per c.cm. while in others there have been found 4,000,000,000 per c.cm. without any curdling. These differences are apparently due to the development of enzymes, and partly to the products of some species neutralising the action of others. The amount of acid present at the time of ordinary acid curdling does not widely vary.

(10) Milk is not necessarily wholesome because it is sweet, especially if it has been kept at low temperatures. At the temperature of an ice box milk may remain sweet for a long time and yet contain enormous numbers of bacteria, among which are species more likely to be unwholesome than those that develop at 20° C.

Although these results show that temperature exerts a selective action on the bacterial flora it must not be forgotten that this may be wholly or partially negatived by a predominance of any particular species in the original milk. For example, milk produced under good conditions and containing less than 10,000 bacteria per c.cm. will very rarely show a predominance of coliform organisms even when incubated at 37° C. The curd produced by this class of milk is almost invariably of the smooth acid type produced by Str. lacticus and seldom gives the gas-blown curd typical of the B. coli group. An examination of the type of curd produced on incubation at 37° C. has

been suggested as a simple method of determining the prevailing type of organisms and will be considered in detail on p. 197.

The development of bacteria in milk at low temperatures was especially studied by Revenal, Hastings and Hammer.[23] Two samples of milk differing widely in bacterial content were stored at 0° C. and the count made at intervals on lactose agar by incubating at 37° C.

TABLE XLV

| Age of Milk, Days. | Dairy Milk. | Barn Milk. |
|---|---|---|
| 0 | 130,000 | 3,500 |
| 6 | 72,500 | 4,050 |
| 15 | 633,500 | 52,900 |
| 20 | 3,230,000 | 1,240,000 |
| 36 | 34,950,000 | 4,800,000 |
| 74 | 91,500,000 | 36,500,000 |
| 106 | 39,750,000 | 192,500,000 |
| 160 | 32,650,000 | 361,000,000 |

That profound modifications had occurred was shown by the fact that at the end of the experiment over 70 per cent of the caseinogen was digested. The total nitrogen decreased, due to liberation of nitrogen in the free state. Pennington[24] also found a digestion of caseinogen when milk was stored at low temperatures, over 50 per cent being digested in five to six weeks at 29°–32° F.

The above results show the importance of storing milk at as low a temperature as is practicable; although 50° F. may be regarded as the critical point for bacterial development, efforts should be made to lower the temperature of milk samples as far as possible if more than a few hours (3–4) elapse between collection and examination. If the samples are immediately surrounded with ice they may be kept for twenty-four hours without altering the significance of the results although the

bacterial count may vary slightly; the direction of this variation will depend upon the condition of the milk when sampled, low counts tending to decrease and high counts to become still higher, thus leaving the general significance unaltered.

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# CHAPTER V

## THE ENUMERATION OF BACTERIA IN MILK

An approximate determination of the total bacteria in milk by plating on solid media has, for many years, been one usually made in connection with the examination of milk, and, although later work has shown that the number so obtained is usually but a small fraction of the total number present, these methods have been generally retained on account of their convenience, and the results are usually described as the total bacterial counts. There has been considerable difference of opinion amongst sanitarians regarding the value of this test, for, whilst some regard the total number of minor importance, others believe that much valuable information can be obtained by this determination alone. The fact that the great majority of regulations for the sale of milk, where regulations have been enacted, contain no other clause with reference to bacteria than a maximum number clause, is sufficient to show the trend of opinion on this subject. Those who deprecate the value of the total bacteria enumeration take the stand that the large majority of the bacteria usually found in milk are harmless saprophytes, and that their determination is more or less a waste of time and labour. Whilst the former statement is undoubtedly true, the latter must be emphatically denied. Until bacteriological technique becomes so developed that routine methods can be applied for the detection of pathogenic organisms, those employed in milk examination must be content with the inferential tests obtained by determination of the saprophytes. As has been shown in the preceding chapter, milk drawn with reasonable aseptic precautions from the udders of cows contains but few bacteria, and, if properly

treated, can be delivered in that condition to the consumer. Laxity on the part of the producer or dairyman by the use of dirty containers or lack of cooling facilities, produces conditions favourable to the development of bacteria for which milk forms an excellent nidus. Once the milk has become contaminated, the organisms multiply very rapidly under favourable conditions, and, by the time the milk reaches the consumer, have become excessive in number. A low bacterial count is an "à posteriori" argument that proper and reasonable care has been exercised in the production of the sample examined, and it

TABLE XLVI

TOXICITY OF MILK (DELÉPINE)

MIXED MILK COMING MORE THAN 40 MILES AND GENERALLY KEPT 24–60 HOURS

| Mean Temp. in Shade, Manchester, during time specimens were kept, Degrees Fahrenheit. | Percentage of Good Specimens. |
|---|---|
| 30–35 | 58 |
| 35–40 | 38.5 |
| 40–45 | 40 |
| 45–50 | 20 |
| 50–55 | .... |
| 55–60 | Nil |

MILK FROM SHORT DISTANCES (LESS THAN 20 MILES) USUALLY KEPT LESS THAN 10 HOURS

| Mean Temp. in Shade, Manchester, during time specimens were kept, Degrees Fahrenheit. | Percentage of Food Specimens. |
|---|---|
| 50–55 | 100 |
| 55–60 | 88.8 |
| 60–65 | 73.2 |
| 65–70 | .... |
| 70–75 | 50.0 |

might fairly be inferred that such milk is less likely to contain pathogenic organisms then one produced by men of careless and slovenly habits. Farmers who take a pride in their produce are more naturally liable to prevent infection of the milk by supervision of their employees, but even if this be not true, it must be admitted that the conditions which tend to keep in check the saprophytes also tend to minimise the relative infectiveness, so that to this extent at least, must credit be given to careful producers and dairymen. Other conditions being equal, the total bacterial count is a measure of relative infectiveness. This statement is supported by the work of Delépine [1] on the toxicity of the Manchester milk supply. He found that "mixed milk . . . showed an increase of virulence on inoculation into guinea pigs in proportion to the mean temperature in the shade in Manchester during the time the specimen was kept." The results are given in Table XLVI, all tuberculous specimens being excluded.

Increased temperature and keeping period result in an increased count so that the above statement can be reduced to one stating that the virulence to guinea pigs was proportional to the bacterial count. Further figures reported by Delépine regarding the relative toxicity of cooled and uncooled milk confirm this.

TABLE XLVII

TOXICITY OF MILK (DELÉPINE)

| | | No. of Samples. | Percentage of Toxic Samples. |
|---|---|---|---|
| 1896–1897. | Unrefrigerated milk... | 141 | 10.7 |
| 1898–1901. | Refrigerated milk..... | 1782 | 2.1 |

Delépine states that "the difference would probably have been greater if the milk had been cooled immediately after milking."

Results reported by the Chicago Department of Health [2] on the relative toxicity of raw and pasteurised milk also confirm this hypothesis.

After this consideration of the "raison d'être" of the bacterial enumeration, the methods by which this is accomplished will now be treated in detail. These may be divided into two groups: (*a*) plating methods and (*b*) direct microscopical methods. The former are based upon the ability of the individual organisms to reproduce at such a rate upon the medium employed as to produce a visible colony within the period of incubation, and the latter upon suitable preparation for direct enumeration under high magnification.

Until within the last few years the former method was the one usually employed, and as it is still in universal use, it will be convenient to discuss it first.

Plain nutrient gelatine prepared with fresh beef infusion was first used with the plate method for the enumeration of bacteria in milk and still enjoys considerable repute with many workers for this purpose, the colonies being usually counted after four to five days incubation at 20° to 22° C. In late years, however, and especially in America, this method has largely been supplanted by the substitution of agar for gelatine and the incubation period reduced to forty-eight hours at blood heat. Although the agar medium does not produce as many visible colonies within the incubation period as the gelatine one, it possesses certain advantages which more than offset this drawback. In routine work it is very desirable that results should be obtained in the shortest possible time, and in this respect the agar medium is decidedly preferable as it reduces the time required by 60 per cent. If necessary the colonies may be counted after twenty-four hours incubation, but the results so obtained do not exhibit the sharp contrasts given by the longer period. Some of the author's results are given in Table XLVIII.[3]

The average of the ratio of the forty-eight hour count to the twenty-four hour count is 3.4, but if the abnormal value of

TABLE XLVIII

EFFECT OF INCUBATION PERIOD ON MILK COUNTS ON STANDARD AGAR

| Sample No. | Incubation Period at 37° C. | | Ratio $\frac{48 \text{ Hours}}{24 \text{ Hours}}$ |
|---|---|---|---|
| | 24 Hours. | 48 Hours. | |
| 684 | 64,000 | 140,000 | 2.2 |
| 685 | 1,500 | 21,000 | 14.0 |
| 686 | 55,000 | 94,000 | 1.7 |
| 687 | 11,600 | 16,000 | 1.4 |
| 688 | 8,500 | 18,000 | 2.1 |
| 689 | 44,000 | 105,000 | 2.4 |
| 690 | 500 | 1,600 | 3.2 |
| 691 | 20,000 | 63,000 | 3.1 |
| 692 | 2,300 | 4,800 | 2.1 |
| 693 | 2,500 | 7,000 | 2.8 |
| 695 | 11,000 | 21,000 | 1.9 |

sample 685 is omitted, it becomes 2.1 with a variation of from 1.4 to 3.2. Conn [4] reports "that in the averages in 28 series of samples submitted to four laboratories, the forty-eight hour count was the larger in 25 cases, smaller in one case, and the

TABLE XLIX

| Sample No. | Bacteria per c.cm. on | |
|---|---|---|
| | Standard Agar 48 Hours at 37° C. | Standard Gelatine 5 Days at 20° C. |
| 1 | 123,000 | 224,000 |
| 2 | 8,000 | 8,600 |
| 4 | 10,300 | 8,800 |
| 5 | 1,300,000 | 1,500,000 |
| 7 | 85,000 | 113,000 |
| 8 | 155,000 | 240,000 |
| 9 | 12,700 | 8,600 |

same in two cases." The averages of the whole series (omitting the samples counting in millions) were 299,000 for the twenty-four hour count and 147,000 for the twenty-four hour count. This gives a ratio of 2.03 : 1. It is obvious that no constant factor can be employed for the ratio of the twenty-four hour count to the forty-eight hour count as this will vary with the bacterial flora. For the same reason the results obtained with the use of different media are not comparable although they usually vary in the same direction. This is well illustrated by the results given in Table XLIX which shows a comparison between standard agar and gelatine.

It will be seen that when the bacterial count is low, the difference between the gelatine and agar count is but small, and, although the gelatine medium usually gives the higher result, this is not an invariable rule; the agar occasionally gives a higher count, but this, in the author's experience, only occurs in a small minority of cases and as the bacterial count increases, the ratio of the gelatine count to the agar count usually becomes greater.

That the addition of 1 per cent of lactose to both nutrient gelatine and agar, favours more rapid reproduction is shown in Table L.

TABLE L

| Sample No. | Standard Agar 48 Hours at 37° C. | Lactose Agar +1 Per Cent 48 Hours. at 37° C. | Standard Gelatine 5 Days at 20° C. | Lactose Gelatine, 5 Days at 20° C. |
|---|---|---|---|---|
| 1 | 123,000 | 180,000 | 224,000 | 240,000 |
| 2 | 8,000 | 8,400 | 8,600 | 8,300 |
| 3 | 12,000 | 11,000 | 6,500 | 12,300 |
| 4 | 10,300 | 11,900 | 8,800 | 13,500 |
| 5 | 1,300,000 | 1,350,000 | 1,500,000 | 1,850,000 |
| 6 | 600,000 | 60,000 | 65,000 | 84,000 |
| 7 | 85,000 | 140,000 | 113,000 | 156,000 |
| 8 | 155,000 | 230,000 | 240,000 | 500,000 |
| 9 | 12,700 | 12,800 | 8,600 | 14,000 |

Heinemann and Glenn [5] investigated the action of dextrose and lactose-litmus agar at 20° C. and 37° C. and concluded that incubation at 20° C. for three days was the most preferable technique as this temperature is less selective in its action than higher ones and so yields more information as to the original flora. After twenty-four hours incubation they found the 37° count to be the higher, but this was reversed after a further twenty-four hours incubation and the difference was still more marked after seventy-two hours. Dextrose and lactose litmus agar gave but insignificant differences in the total count but the former showed a decidedly higher percentage of acid colonies, due, it is suggested, to colonies of the B. aerogenes type becoming red only temporarily and finally assuming a blue colour. For this reason Heinemann and Glenn prefer dextrose to lactose. The high counts obtained by these observers seem to indicate that the samples had been kept for some time and that considerable reproduction had taken place. This possibly had an effect on the results obtained. For example: Str. lacticus, which is usually abundant in stale milk, grows well at 20°, but at 37° produces colonies in forty-eight hours that are barely visible even with the aid of a low-power magnifying glass and are usually overlooked when the medium is tinted with litmus.

The Committee on Methods of Milk Analysis appointed by the American Public Health Association to investigate the various details of the plate method using an agar medium reported as follows (Am. J. of Pub. Hyg., 18, 431).

*Acidity* (to phenolphthalein at boiling point). Of the acidities+0.5, +1.0, +1.5 and 2.0, an acidity of +1.5 per cent gave the best results.

*Lactose.* 0, 1, 2, 3, and 4 per cent of lactose was tried at incubation temperatures of 20° C. and 37° C. At 37° C., they found that the medium free from lactose was preferable, but at 20° C. the one containing 1 per cent of sugar was the best.

*Whey, Plain, and 4 Per Cent Lactose Agar* media were com-

pared in 74 tests. In 28 tests ordinary agar gave the best results, whey agar in 24 tests, and lactose agar in 22 tests. They found that whey agar favoured the growth of lactic acid organisms and ordinary agar of organisms other than lactic acid producers.

*Agar and Gelatine.* Litmus lactose agar at 37° C. was compared with litmus lactose gelatine at 20° C. in 25 tests: of these gelatine gave higher results in 18 tests and agar in 7. Where gelatine showed the higher count the percentage difference was much greater than where agar showed the higher numbers. It was also found that the differentiation of species was much better on gelatine but that there was a considerable loss of plates with this medium.

Both media were used at 20° C. in 24 tests and in this series gelatine was the better in 14 and agar in 10 samples. When beef peptone gelatine at 20° C. with seventy-two hours incubation was tried against beef peptone agar at 37° C. with twenty-four hours incubation, gelatine gave the higher count in 18 tests, agar in 4 tests, and in one test they gave identical results. The total gelatine count, however, was more than double that on the agar plates. The standard method for the examination of milk as adopted by the American Public Health Association in 1912 was the plate method with a plain agar medium of +1.5 per cent acidity, made with beef infusion and 1 per cent each of peptone and dried agar. The 1916 report recommended certain alterations; concentrated beef extract, 3 gms. per litre, was substituted for beef infusion and the acidity was reduced to +1.0 per cent: the quantity of peptone was reduced to 5 gms. per litre and the agar increased to 1.2 per cent of the dried material. Although the author has not compared fresh beef infusion media with similar media prepared with Lemco for the enumeration of bacteria in milk, his experience with water was that the Lemco media invariably gave higher and more consistent results. The reason for variable results with beef infusions or decoctions lies in the difficulty in obtaining solutions of even approximately con-

stant composition and in the variable quantity of alkali required for the adjustment of the acidity.

Clark [3] has pointed out that the method of adjusting the acidity of media, as recommended in the standard methods of analysis, is not scientific in principle and that it does not ensure a constant hydrogen ion concentration. Various batches of media prepared by different workers and adjusted to an acidity of +1 per cent by the standard method (titration of the boiling medium with alkali using phenolphthalein) were found to have very different H ion potentials when tested by the electrical method. No results are given by Clark as to the effect of this variation on the bacterial reproduction in these media but the comparative experiments of a group of New York bacteriologists indicate that any variation due to this cause is insignificant and can safely be ignored. In these experiments media were prepared by four laboratories and supplied to Dr. Conn, of Middletown, Conn., who plated out two samples of milk on each medium in triplicate. The results were as follows:

| Medium. | Borden. | North. | Board of Health. | Lederle. |
|---|---|---|---|---|
| Sample 1... | 12,000 | 15,000 | 14,000 | 13,000 |
| Sample 2... | 305,000 | 290,000 | 280,000 | 279,000 |

Three of the above media gave an acidity of +1.0 per cent, as determined by Conn, and the fourth +0.9 per cent. These results show that media prepared in various laboratories according to standard methods give results as close as can be expected from a consideration of the technique.

The technique of bacterial enumeration in milk was carefully investigated by the New York group of bacteriologists above referred to and the results summarised by Conn.[4] Samples of various grades of milk and cream were prepared by Conn and duplicate samples forwarded to the various laboratories partaking in the work. As the samples invariably included duplicate samples under different numbers, each sample was

not only examined in four laboratories but each laboratory was unknowingly checking the accuracy of its own work. The various points investigated were as follows:

**1. Method of Inoculation.** Three methods were employed: (*a*) Measurement of the sample into plates and pouring the agar from flasks, (*b*) measurement into plates but pouring the agar from tubes, and (*c*) inoculation of the tubes and pouring into plates after rolling. The results obtained show the slight superiority of the tube inoculation method but the advantage is so slight as to be of no real importance. In the few cases where methods (*a*) and (*b*) were compared, (*a*) gave higher results though there is no manifest reason why this should occur. In laboratories where large numbers of samples are examined the slight superiority of the tube inoculation method is more than offset by the economy in material and labour effected by the use of the flask method. The author's experience has been that, although the time required for plating samples was not very much reduced, the preparation of media was greatly facilitated and the cost reduced.

**Composition of Media.** In one series three different media were used (*a*) standard agar (beef bouillon with the addition of 1 per cent agar and peptone and adjusted to +1.5 per cent acidity), (*b*) standard agar with the substitution of Liebig's extract for beef infusion, and (*c*) agar prepared with beef extract but containing only one-twelfth the quantity in (*b*) and having an acidity of +0.3 per cent.

The results showed that

In 30 samples (*a*) medium gave the highest count.
In 27 samples (*c*) medium gave the highest count.
In 20 samples (*b*) medium gave the highest count.

So far as the actual numbers were concerned the differences were of no real significance so that, in this respect, the media were of equal value. The size of the colonies on (*c*) medium was generally small and rendered accurate counting more difficult. Against this disadvantage must be placed the decreased

trouble experienced with spreaders. Observations for spreaders indicated that 128 were found with (*a*) medium, 21 with (*b*) medium and 23 with (*c*) medium. On the whole, it would appear that the (*b*) medium was the most satisfactory.

**Uniformity of Technique.** The several series of comparative examinations produced some interesting data on the influence of technique. In the first series when each laboratory used the technique as previously developed in that laboratory, the results on duplicate samples showed a variation factor of from 1.3 to 43.2 with an average of 6.2. The variation factor was obtained by dividing the highest result by the lowest. Duplicate analyses in each laboratory also showed variations, the average factors varying from 2.1 to 4.8 with a general average of 3.7.

In a second series of tests the various laboratories all employed identical technique as to shaking of sample, diluting, pipetting, inoculating, and counting of plates. As it was found in the first series that one laboratory employed a magnifying lens for counting plates and another the naked eye, it was decided to use a standard lens in all laboratories and to determine the personal error in counting by an exchange of incubated plates. The results showed that the personal error may be a serious one, for, although the variation in duplicate counts of identical plates was usually small, the extreme variation was nearly 100 per cent. In this series the average variation in each laboratory was from 1.6 to 2.2 with a general average of 1.8.

A five-day count was also compared with the two-day count and, although the results were usually higher they were not uniformly so. There seems to be no apparent advantage attainable by prolonging the incubation period beyond the usual forty-eight hour period.

In the third series the effect of agitation, amongst other points, was determined, and although the results are not conclusive they indicate the importance of standardising this portion of the technique. In the third and fourth series the plate

method of enumeration was also compared with the direct microscopical method of Breed but this will be dealt with later.

From a consideration of this work Conn pointed out that variations in technique are much more important than the composition of the medium, and that variations in results may reasonably be expected, even under the best conditions due (1) to clumping of the bacteria, and (2) to the bacteria being in non-uniform suspension and not in solution. These two factors render it improbable that two small samples will contain equal numbers of organisms, and the lower the total number of bacteria the greater will this divergence become. Conn expressed the opinion that "individual counts under the best conditions are subject to considerable variation and that no single individual count can be relied upon." . . . "It is not possible to rely upon a greater accuracy than 100 per cent even when the average of more than one sample is obtained, although most of the results fall considerably below this limit."

During 1915 the author made a series of duplicate examinations of milk by plating one of the routine samples in duplicate daily; in this series plates containing $\frac{1}{100}$ c.cm. and $\frac{1}{1000}$ c.cm. were inoculated and counted with a low-power glass after forty-eight hours incubation at 37° C. Porous covers were used to prevent loss of plates by spreaders. In 142 samples the difference between duplicate determinations varied from zero to 464 per cent with an average variation of 24.7 per cent. Expressed as a variation factor the average was 1.25 (1.247) with a maximum of 4.64. The bacterial count varied from 1600 per c.cm. to 1,200,000 per c.cm. and it was with the best grade milks, i.e., those containing less than 10,000 per c.cm., that the variations were the largest. This was anticipated from a consideration of the frequency distribution in the largest amount of sample plated and could have been reduced by inoculating larger quantities. This was not done because the labour involved in so treating all samples, when but very few were of this grade, was not justified by the increased precision so obtainable, for whether a sample contains 1600 or 5000 organisms

per c.cm. has no real bearing on its hygienic quality. This series of comparative results is not so important as that reported by Conn because of the psychological factor; both the person plating out the samples (A. J. S.) and the one counting the plates (J. R.) were aware that these determinations were being made, and although every endeavour was made to honestly record the actual conditions found, it is recognised that the results are subject to these limitations.

The detailed technique for the plate method as adopted by the American Public Health Association in 1916 is as follows:

*Dilutions.* For samples of unknown character dilutions of 1 to 100, 1 to 1000, 1 to 10,000 shall be made, using sterile water and pipettes after the ordinary method. In case the character of the milk is known, less than three dilutions may be made; but in no case shall less than two plates for each sample be made. Grade A,* or its equivalent, should be plated in duplicate, and a dilution lower than 1 to 100 may be used.

*Shaking.* Samples must be shaken twenty-five times. Shaking is defined as meaning a rapid up and down motion with an excursion of not less than 1 foot.

*Pipettes.* Pipettes must be made to deliver between graduation marks, not simply to deliver.

*Pouring Plates.* The melted agar must be poured promptly after measuring out the proper quantities of milk. Not more than twelve plates must be allowed to accumulate after the distribution of the milk into the plates before pouring the agar.

*Incubation and Counting.* One standard temperature only is recognised—forty-eight hour incubation at 37° C.

If possible count those plates containing between 30 and 200 colonies. If there are none such, count those plates containing nearest to 200 colonies. The whole number of colonies on the plate shall be counted where the plates contain less than 200 colonies.

* Milk usually containing less than 10,000 bacteria per c.cm.

*Counting Lens.* The lens recommended by the Committee in 1914 is more fully defined. It is known as Engraver's lens No. 146, Bausch & Lomb catalogue. It is designated as $3\frac{1}{2}$X, its magnification being $2\frac{1}{2}$ diameters. Persons who are near-sighted should wear their ordinary glasses while using this lens. Farsighted persons should use the lens without their glasses.

**Direct Methods.** The direct methods of enumeration of bacteria in milk are of comparatively recent development; in these the milk or centrifugalised sediment is smeared over a slide, and, after suitable staining, examined under a high-power objective and the bacteria counted. The direct method as modified by Slack [8] is as follows. Two c.cms. of the sample, after thorough shaking, are inserted into special tubes with rubber stoppers at each end, and centrifugalised for ten minutes at 2500 revolutions per minute in a special apparatus. This apparatus is a modification of the one used by Stewart of Philadelphia for leucocyte estimation, and consists of an aluminium disc and cover 10 inches in diameter and $\frac{3}{8}$ inch in depth, fitted to hold twenty tubes arranged radially. This apparatus is manufactured by the International Instrument Co., of Cambridge, Mass., and can be used with the usual electrical centrifuge. After centrifugalising, the tubes are carefully removed, and, to obtain the sediment with the least disturbance, the tube is held with the cream end downwards, whilst the cream layer is removed by means of a platinum loop. The milk is then carefully poured out without permitting air bubbles to ascend the tube, and finally, with the tube in the same position, the other stopper is removed and the sediment is smeared on a glass slide with the aid of a drop of sterile water. An area of 459 cms. is a convenient one and squares of this size may be marked off on a strip of glass with a blue grease pencil. The smear is dried, fixed by heat, and stained with methylene blue. The specimen is then examined under a $\frac{1}{12}$ inch oil immersion lens and the organisms counted. Each coccus, bacillus, diplococcus, or chain represents a colony on the 1–10,000 plate of the same sample when grown on agar for twenty-four hours at 37° C.

This factor of 10,000 was modified later to 20,000 in order to correspond to the forty-eight hour incubation period. Whilst it was not claimed that the whole of the bacteria are contained in the sediment, it was asserted that in 99 per cent of the samples a representative number is so precipitated, and that this number bears a fairly constant relation to the bacterial count as determined by plating on agar.[9]

Slack, in a series of over 2200 samples, compared the results obtained by the centrifuge and plate methods (twenty-four hours at 37° C.) and an error of less than 1 per cent was made in passing as below 500,000 bacteria to the cubic centimetre, milks which the plates showed to be above this limit.

This method has also been examined by Gooderich[10] who reports very favourably upon it and remarks that very little improvement can be made upon the factor $2\times10^4$ (20,000) for converting the microscopical results to the forty-eight hour count on agar. He reports the limits for the factor as being from $0.66\times10^4$ to $6.0\times10^4$. With a standard of 50,000 bacteria per c.cm. he found that the direct method wrongly passed 8.6 per cent, and wrongly condemned 8.9 per cent, but that when the standard was raised to 100,000 these figures were reduced to 1.4 and 4.3 per cent, respectively. In considering these results it is important to note that all the determinations were made on samples secured from the University Stock Farm. The variations in bacterial content of such samples would not be nearly so great as is met with in routine work on various market milks of unknown origin, with the consequence that the errors would be minimised. The small variation in the counts is clearly indicated by the fact of the mention of only a 1–1000 dilution being used for plating. Such a procedure is impossible in routine work on market samples in which the count may vary from a few hundreds to 5,000,000 or even more. In view of the excellent results obtained by Gooderich, the writer experimented with this method, although a consideration of the fundamental principles did not lead to an anticipation of a high degree of accuracy[3]. If the results were to correspond with the

usual plate count it was essential that a constant proportion of the bacteria capable of development on agar in forty-eight hours at 37° C. must be precipitated during the process of centrifugalisation. A portion of the bacterial flora of milk, however, does not produce visible colonies on agar under the usual conditions, so that either these organisms must remain in suspension or the error due to them be counterbalanced by some other factor.

No difficulty was found with the technique until the microscopical examination was made. The representative field in which the organisms were to be counted was difficult to find owing to the widely differing content of various fields. In order to minimise this source of error ten fields were taken at random and the average calculated.

In a series of market samples, for which the standard was 500,000 bacteria per c.cm. not a single sample was condemned which passed the plate method; on the other hand, 17 per cent were passed which were condemned by the plate method. According to these results the direct method outlined above would not be oppressive on the milk producer, and its adoption would be tantamount to lowering the standard. In this series the factor (*c*) for the conversion of microscopic counts to plate counts varied within very wide limits, viz., from $0.4\times10^4$ to $33.0\times10^4$, and the author is convinced that this is largely due to the difficulty found in obtaining an even distribution of organisms on the slide. Two observers obtained widely varying results from the same slide; a condition fatal to accuracy. Breed,[11] in 1911, improved this method by making a direct smear of the milk and thus eliminating the centrifuge with its many unknown factors. Breed's method consists essentially in spreading a small volume of milk over a marked area and examining under a high-power objective after washing out the fat followed by suitable staining. Skar,[12] in 1912, independently developed a similar method which differs only in the manner of staining and in allowing the fat to remain in the smears. Rosam's method[13] differs essentially from Skar's

method only in the method of smear examination: these are made on a cover glass and examined whilst wet.

In some of the comparative experimental work reported by Conn and discussed on page 123, a series of bacterial counts was made by Breed and this was supplemented in a further series by the inclusion of Brew, a co-worker with Breed. These experimenters made microscopical counts on the samples plated by other observers, and Conn[14] considered that when the groups of organisms only were counted, the count agreed somewhat closely with the plate count. When raw market milk was examined, the variations found were generally not greater than the differences between the plate counts in various laboratories, but for high-grade raw milk and pasteurised products it is comparatively useless. The details of Breed's process are as follows: 0.01 c.cm. of milk, from a well-shaken sample, is measured out by means of an accurately calibrated special pipette and deposited on a glass slide on which an area of 1 square centimetre has been previously marked out. The drop is evenly smeared over this area with a stiff needle and gently dried at about 50° C. The slide is then placed in a Coplin staining jar containing xylol or gasoline to remove the fat, and, after drying, fixed in alcohol (70 to 95 per cent). Immediately afterwards the smear is stained with 1 per cent aqueous methylene blue and, finally decolourised to a light blue in 95 per cent alcohol. The microscopical examination is made with a $\frac{1}{12}$ inch oil immersion objective. In order to find the factor for converting the number of organisms per field into organisms per cubic centimetre the diameter of the field is determined with a stage micrometer. The factor is then calculated from the formula:

$$\frac{x}{\pi R^2} \times 100 = y,$$

where $y$ is the factor sought, $x$, the area of the smear in square millimetres, and $R$ the radius of the field.

In practice it is convenient to pull out the draw tube until

the area of the field is of such a value as will give a value to $y$ having as many ciphers as possible. The following are the most satisfactory.

When $R=0.080$ m.m., $y=500{,}000$

When $R=0.089$ m.m., $y=400{,}000$

When $R=0.101$ m.m., $y=300{,}000$

When the desired result is obtained the position of the draw tube is noted and always set at this point in future examinations. In order to get results comparable with the plate method, only the groups or clumps, together with isolated bacilli are counted; individual cocci, diplococcus or streptococcus chains, and rod forms where the plane of division shows clearly, are counted as individuals. The number of fields to be examined must be determined by the frequency of the organisms. It is obvious that with a factor of 300,000 to 500,000, this method is of the greatest advantage when the count averages one clump or more per field; with high-grade milks under 10,000 bacteria per c.cm. the number of fields to be examined would be so large, if reasonable precision is to be obtained, as to consume as much time as the plate method. Dead bacteria are counted with the living, so that this process is not applicable to pasteurised products; it would, however, be of advantage in determining the quality before pasteurisation. A collateral advantage of this method is that in addition to the quantitative estimation of the bacteria, a cell count can be made at the same time and information obtained regarding the bacterial flora.

As an indirect method for estimating the number of bacteria, Barthol,[15] in 1908, suggested the employment of methylene blue. It was found by Barthol and confirmed later by Jensen and Muller, that the time required to decolourise methylene blue bears a relationship to the number of bacteria present. Fred [16] showed that 21 of 23 species of milk bacteria were capable of reducing methylene blue and that each species has a

different coefficient of velocity; the velocity of reduction was a linear function of the temperature (up to 37° C.) and, finally, ceased with exhaustion of the medium. It was formerly suggested that the reduction of methylene blue in this "slow reductase test" as it is usually termed, was due to enzymes present in the intramammary milk, but it is now generally held that such milk does not contain reducing substances and that the reduction is due to intra and extra cellular products of bacterial origin.

Fred [17] in an examination of 200 samples of milk by this method (adding 1 c.cm. of a 0.05 per cent solution of pure methylene blue in 0.4 per cent saline to 10 c.cms. of milk and holding at 40° C.) found that the time required for reduction was proportional to the bacterial count. His figures are given in Table LI, each group representing the average of 20 samples.

TABLE LI

| Group Number. | Average Number of Bacteria per c.cm. | Average Time of Reduction in Hours. |
|---|---|---|
| 1 | 29,647 | 11.9 |
| 2 | 73,587 | 9.7 |
| 3 | 160,150 | 9.5 |
| 4 | 283,250 | 8.0 |
| 5 | 548,300 | 7.8 |
| 6 | 1,016,600 | 4.7 |
| 7 | 1,469,650 | 3.1 |
| 8 | 2,505,000 | 2.7 |
| 9 | 4,690,000 | 1.5 |
| 10 | 8,624,800 | 1.0 |

Barthol [18] found that samples containing more than 10,000,000 bacteria per c.cm. and 50 per cent of those containing 4–10 millions per c.cm. reduced within one hour. He concluded that 10 millions per c.cm. was the lowest limit that could be estimated by this method and that below this limit there is no

relationship between the number of bacteria and the time required for decolourisation.

The author examined a number of milks by this test in 1914 but was unable to find any merit in it. Almost all the samples failed to decolourise in the six hours that were available for observation under ordinary laboratory conditions, and they had generally showed reduction by the following morning (twenty-one hours). As over 90 per cent of these samples contained less than one million bacteria per cubic centimetre these results are not inconsistent with Fred's (*vide supra*), but as the time of reduction could only be determined within wide limits no real information could be deduced as to the bacterial condition of the sample, except that it did not contain very excessive numbers. Samples that were allowed to stand and develop large numbers of organisms showed small reduction periods and it would seem that it is in the detection of such milk that the chief value of the test lies.

A further rapid indirect method that has been suggested for the approximate determination of the bacterial content of milk is the estimation of the acidity. Milk almost invariably contains acid-producing organisms, and as these find milk an excellent medium for development it would seem to be logical to assume that the determination of the products of bacterial metabolism would bear some relation to the number of organisms present. Fred (*vide supra*) determined the acidity of 200 samples of milk and arranged the results into groups of 20 according to the bacterial count. His results are given in Table LII.

Fred is of the opinion that the acidity determination serves a useful purpose in indicating to some extent the proper dilutions to be used for the bacterial counts, and adds that "the relationship to the number of bacteria is only approximate." Russell and Hastings have also suggested using this test as a guide to the dilutions to be made in the plate method and advise 10, 100, and 1,000 dilutions for acidities under 0.2 per cent and 1,000, 10,000 and 100,000 for acidities over 0.2 per cent.

TABLE LII

RELATION OF ACIDITY TO BACTERIAL COUNT (FRED)

| Group Number. | Average Acidity as Lactic Acid. | Number of Bacteria per c.cm. |
|---|---|---|
| 1 | 0.189 | 29,647 |
| 2 | 0.188 | 73,587 |
| 3 | 0.183 | 160,150 |
| 4 | 0.201 | 283,250 |
| 5 | 0.192 | 548,300 |
| 6 | 0.205 | 1,016,600 |
| 7 | 0.206 | 1,469,650 |
| 8 | 0.212 | 2,505,000 |
| 9 | 0.231 | 4,690,000 |
| 10 | 0.250 | 8,624,000 |

The author, during 1914 and 1915, determined the acidity and bacterial count of a number of the samples received for routine examination with the following results:

TABLE LIII

RELATION OF ACIDITY TO BACTERIAL COUNT (AUTHOR)

| Number of Samples. | Acidity. | | Bacterial Count 48 Hours at 37° C. |
|---|---|---|---|
| | Degrees. | Lactic Acid, Per Cent. | |
| 34 | 14 | 0.126 | 203,000 |
| 67 | 15 | 0.135 | 332,000 |
| 102 | 16 | 0.144 | 282,000 |
| 144 | 17 | 0.153 | 289,000 |
| 186 | 18 | 0.162 | 232,000 |
| 185 | 19 | 0.171 | 212,000 |
| 120 | 20 | 0.180 | 175,000 |
| 32 | 21 | 0.189 | 408,000 |
| 28 | 22 | 0.198 | 397,000 |
| 9 | 23 | 0.207 | 541,000 |

These results show no definite relationship between the acidity and the bacterial count until the acidity approaches 0.20 per cent (22°), and in this respect, are confirmatory of Fred's results. Only 9 samples out of a total of 917 exceeded 22° acidity and it became obvious that the acidity determination even as a guide to the best dilutions to employ in plate work did not give information commensurate with the labour involved. For pasteurised and heated milk the acidity estimation is of even less value than for ordinary raw milk owing to the change in acidity acused by the heating processes.

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# CHAPTER VI

## EXCREMENTAL ORGANISMS

The estimation of typical excremental organisms in milk is of considerable value because of the general absence of these bacteria in intra-mammary milk; they indicate, therefore, the amount of care exercised in the production and handling of the milk in a rather better manner than the determination of the *total* number of organisms, but as milk drawn under the best conditions is never absolutely free from excremental organisms, this advantage is merely relative.

The estimation of the bacteria usually regarded as indicative of manurial pollution has not in the past been developed to full advantage because of the somewhat elaborate technique involved, and also because some sanitarians have regarded the excremental bacterial content as being more determined by duration and conditions of storage than by the original pollution. It would, undoubtedly, be of great advantage if some method could be found of determining the manurial pollution of a sample at the time of milking, not only because it would yield precise information as to the condition requiring correction, but also on account of the possible association of tubercle bacilli with the fæcal bacteria. Tubercle bacilli grow so slowly in milk in comparison with the typical excremental organisms that any inferential value associated with the determination of the latter is rapidly nullified by the conditions usually obtaining in the marketing of milk.

The organisms commonly used as indicators of manurial pollution are B. coli, B. enteritidis sporogenes, and Streptococci, and of these B. coli is probably the most important and the most easily estimated. English bacteriologists have, on the whole,

devoted more attention to these estimations than their American confrères, but neither have studied them as fully as they deserve and it is to be hoped that this condition will soon be rectified.

These organisms will now be treated in detail.

**1. B. Coli.** The term B. coli in these pages is used to signify the general group of aerobic, non-sporulating organisms that ferment lactose with the production of acid and gas, and not one particular member of the group, such as B. coli communis, having certain specific characteristics in addition to the generic ones just described. Many attempts have been made to regard certain members of this group as being more significant than others-but this has been a comparative failure when viewed by the light of later experience.

MacConkey [1] reported upon the biochemical characters of a number of members of the B. coli group, isolated from milk and from the fæces of cows, and classified them into four groups according to their action on saccharose and dulcite. The results are given in Table LIV.

TABLE LIV

| | Milk. Per Cent. | Cow's Fæces. Per Cent. |
|---|---|---|
| Saccharose+dulcite+ | 32.7 | 47.9 |
| Saccharose−dulcite+ | 39.2 | 25.0 |
| Saccharose+dulcite− | 19.6 | 12.5 |
| Saccharose−dulcite− | 8.4 | 16.6 |

MacConkey suggested that these groups should be further subdivided according to the ability to ferment adonite and inulin, the Voges and Proskauer reaction, and the motility. In 1909 he reported the characteristics of colon organisms isolated from animal and human fæces and arranged the grouping in accordance with the subdivision.[2] As this further division has not been generally adopted, the results have been

rearranged into the four general groups in Table LV and Orr's results [3] added for comparison.

TABLE LV

| | MacConkey. | | Orr. | | | |
|---|---|---|---|---|---|---|
| | Human Fæces. | Animal Fæces. | Milk from Cow-shed. | Milk from Retailer. | Milk from Con-sumer. | Manure. |
| | Per Cent. | Per Cent. | Per Cent. | Per Cent. | Per Cent. | Per Cent. |
| Saccharose+dulcite+. | 32.2 | 48.1 | 28.5 | 26.5 | 26.1 | 18.7 |
| Saccharose−dulcite+. | 27.0 | 34.3 | 13.8 | 10.4 | 12.8 | 35.4 |
| Saccharose+dulcite−. | 4.5 | ..... | 43.9 | 39.1 | 41.1 | 33.4 |
| Saccharose−dulcite−. | 28.0 | 8.4 | 12.6 | 20.4 | 16.7 | 8.4 |
| Other strains..... | 8.3 | 9.2 | 1.2 | 3.6 | 3.3 | 4.1 |

The results of Rogers et al.,[4] who investigated 107 colon organisms obtained from milk products, and some unpublished ones of the author on the biochemical characters of coliform organisms obtained from 226 samples of milk, are given in Table LVI.

TABLE LVI

| | Rogers et al. Per Cent. | Author. Per Cent. |
|---|---|---|
| Saccharose+dulcite+..... | 24.3 | 46.5 |
| Saccharose−dulcite+..... | 14.9 | 8.4 |
| Saccharose+dulcite−..... | 37.4 | 36.3 |
| Saccharose−dulcite−..... | 23.4 | 8.8 |

The author's results, obtained with samples of the Ottawa milk supply, are somewhat in accordance with Orr's results as regards the predominance of saccharose fermenters, but show a larger proportion of dulcite fermenters. This predominance of saccharose fermenters accords with the results recorded for

animal fæces and would seem to differentiate between animal and human pollution, but as the difference is one of degree only and is not specific, no definite significance can be attached to it. Although a large amount of work has been done on the separation of the colon group of organisms, no test or combination of tests has been evolved that would indicate that any one subgroup is more typical than another, and it must, therefore, be borne in mind that to designate any organism as being *typical B. coli* because it possesses certain biochemical and morphological characteristics is a purely arbitrary and empirical procedure. Moreover, these organisms are not to be regarded as having immutable properties like chemical compounds, but to form involution and mutation varieties according to the environment.

Milk, even when produced under the best conditions, is never quite free from B. coli, but if reasonable precautions are taken, this group should not be present in 25 c.cm. quantities of byre milk. Even after bottling and delivery to the purchaser milk can be produced that will average less than two B. coli per cubic centimetre, even during the summer months. This is exemplified in Table LVII.

TABLE LVII

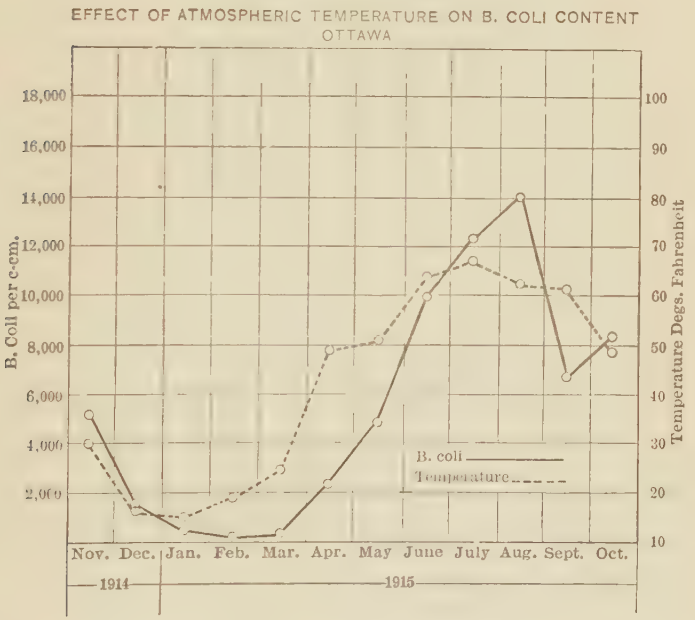
BACTERIA AND B. COLI IN CERTIFIED MILK (AUTHOR)

| Month. | Mean Bacterial Count per c.cm. | Mean B. Coli per c.cm. |
|---|---|---|
| May | 5,700 | 1 |
| June | 10,900 | 2 |
| July | 5,000 | 0.1 |
| August | 4,500 | 0.8 |
| September | 5,500 | 1.4 |

When milk is kept at a temperature not exceeding 45° F. the B. coli do not increase (*vide* p. 104) and this temperature may, therefore, be regarded as the critical anabolic temperature. Above this point they multiply rapidly and in summer

the B. coli content of milk must be regarded as due more to reproduction than to original contamination. Diagram No. III, which shows the B. coli content of the Ottawa raw milk supply compared with the mean atmospheric temperature, demonstrates very clearly the effect of temperature. In the autumn months the curves do not correspond because the mode of the B. coli curve is lowered during the hot summer months

DIAGRAM No. III




by artificial cooling of the milk and the temperature of the milk is, consequently, not proportional to the atmospheric, but it is evident that artificial cooling is abandoned before the natural agencies become entirely operative. It is also interesting to note that after the very cold winter weather the B. coli content does not increase until the mean atmospheric temperature exceeds the critical temperature.

**Estimation of B. Coli.** The methods in vogue for the estimation of B. coli fall into two groups, (1) enrichment methods and (2) plate methods.

*Enrichment Methods.* In the enrichment methods, varying quantities of the sample are inoculated into liquid media and incubated, the media being subsequently examined as to the presence or absence of B. coli. In this test a carbohydrate is usually employed that is fermented by B. coli with the production of gas and special tubes are used in which this gas is trapped and retained as visible evidence of fermentation. On account of the economy of space a small inverted tube contained in a larger ordinary culture tube (Durham's tube) is now in almost universal use in the fermentation process. As in water examination, there are a number of points in connection with this test that require consideration. The first is the composition of the medium to be employed. If the results are to be based on the presence or absence of gas in the tubes, it is evident that lactose and not dextrose must be the carbohydrate employed as there are other groups than B. coli that ferment the latter sugar. The nitrogen requisite for bacterial reproduction is usually supplied by the addition of peptone, although this may be partially displaced by sugar-free beef infusion or extract. Potassium chloride is also a desirable constituent (Chamot and Sherwood). Such a medium will give gas formation even with attenuated B. coli, and, if only vigorous forms are desired to be estimated the medium can be prepared with a base of fresh ox bile instead of water. There is considerable evidence, however, that the lactose ox-bile medium inhibits the growth of a number of vigorous forms of B. coli in addition to the attenuated ones and for this reason the fresh bile medium is often regarded with disfavour. MacConkey's medium, containing 0.5 per cent of bile salt, may also be used and in this case the results will usually be intermediate between those obtained with lactose broth and lactose bile. The main objection to lactose broth is the excessive number of anomalies caused by the overgrowth of other organisms. Aciduric bacilli

occasionally reproduce so rapidly in the lower dilutions as to prevent the growth of the coliform bacteria and so give a negative gas test when a much higher dilution of the same sample shows copious gas formation.

The usual amounts of lactose and peptone employed in the fermentation test are 1 per cent of each, but Chamot and Sherwood [5] have shown that a lactose content of 0.6 per cent produces equally satisfactory results as 1.0 per cent. Under 0.6 per cent the results were irregular and the total volume of gas small, whilst quantities much exceeding 1.0 per cent retarded the rate of gas formation. With normal acidities they found that the total gas volume was proportional to the concentration of the nitrogen whether present as peptone, beef extract or infusion. With increasing amounts of peptone the increase in gas volume was rapid until 4.0 per cent was reached and when both final volume and rate of production were considered, it was found that a concentration of 3.0 to 4.0 per cent was the optimum. Potassium chloride (0.6 per cent) hastened gas formation and was found superior to phosphates and other salts. The concentrations finally recommended were lactose 0.8 per cent, peptone 3 to 4 per cent, KCl 0.6 per cent, and the reaction +1.0 per cent. With lactose bile the nitrogen content should be sufficient with the addition of only 1.0 per cent of peptone, but in other media the higher amount should be employed. For the concentration method the author uses ordinary lactose broth or lactose bile salt broth in preference to lactose bile on account of the irregularities often found with lactose bile and due to the variations in composition.

The number of tubes to be employed in order to obtain reasonably precise results is the second point for consideration. It has been usual to use such dilutions of milk that the quantities represent decimal fractions of 1 c.cm. and to endeavour to obtain at least one positive and one negative result. Although, in many instances, no attempt has been made to convert such positive and negative findings into mathematical expressions, others have attempted to do so by taking the

reciprocal of the lowest quantity showing a positive result as representing the number of B. coli per cubic centimetre. Thus, 0.1 c.cm.+, 0.01 c.cm+, 0.001 c.cm.−, was expressed as 100 B. coli per cubic centimetre. When the average of a number of samples from one source is calculated by this method (Phelps [6]) an accurate result is obtained providing the series is fairly large (about 25), but McCrady [7] has shown that for individual samples such assumptions are far from accurate. McCrady calculates from the theory of probabilities that the most probable number of B. coli present per cubic centimetre, if the above result were obtained, would be 230 and not 100 as assumed. It is possible that *any* number of B. coli per cubic centimetre would produce this result and, in order to reduce the range of possibilities and sharpen the probability curve, it becomes necessary to employ more than one tube of each dilution. The greater the number of tubes used the greater is the precision obtained. With a milk of unknown origin that may contain up to 100,000 B. coli per cubic centimetre it is obvious that even if only three tubes of each dilution are used the total number of tubes for each sample becomes so great as to be cumbersome. For this reason the tube method of estimating B. coli in milk cannot be recommended.

The third point for consideration is the method of recording the results. If desired, all tubes showing gas may be plated out on rebipelagar or litmus lactose agar and the red colonies so obtained put through confirmatory tests, but as such a procedure requires much time and labour it will be found more convenient and fairly accurate to record all tubes as positive that show more than 5 per cent of gas. Anomalies at the higher end of the series should be ignored as they are probably the result of overgrowths, but those at the lower end should be corrected by moving the lower positive results to the next higher dilution; thus, 1.0 c.cm.−, 0.1 c.cm.+, 0.01 c.cm.+, 0.001 c.cm.+, should be recorded as 1.0 c.cm.+, 0.1 c.cm.+, 0.01 c.cm.+, 0.001 c.cm.+, but 1.0 c.cm.+, 0.1 c.cm.+, 0.01 c.cm.−, 0.001 c.cm.+, should be recorded as 1.0 c.cm.+, 0.1 c.cm.+, 0.01 c.cm.+, 0.001 c.cm.−.

*Plate Methods.* Quite a number of solid media have been suggested for the isolation and enumeration of B. coli and allied organisms and of these the most useful are Endo's medium (fuchsin sulphite agar), Drigalski and Conradi's medium (nutrose agar), æsculin bile salt agar, and rebipelagar (neutral red bile salt agar). On account of the difficulties connected with the preparation and use of the first two media the author prefers the latter two. These are easy to prepare (see appendix p. 207) and may be used in exactly the same manner as ordinary nutrient agar or gelatine. The Committee on Standard Methods of Milk Analysis of the American Public Health Association investigated the latter two media and reported in favour of the æsculin medium. They found more bacteria of the B. coli group on rebipelagar in nearly every instance but this was due to the difficulty in deciding which were the coliform colonies on the æsculin medium. Of more than fifty colonies subcultured from the neutral red medium only 67 per cent were found to be B. coli or B. ærogenes (B. lactis ærogenes) whereas all the dark colonies from the æsculin medium were of the B. coli family. Savage [9], from his experience with æsculin agar and rebipelagar, as compared with lactose bile salt broth, has expressed the opinion that both media are equally useful but inferior to L. B. B. tubes on account of the difficulty in arriving at accurate estimations of the numbers by direct plating. The author has had very little experience with æsculin agar, but the extended observations that he has made with rebipelagar do not entirely agree with the above results. A series of comparative experiments on 100 samples with rebipelagar and lactose bile salt broth gave the following results, gas formation being regarded as evidence of the presence of B. coli in the tube series without confirmation.

| Medium. | B. coli per C.cm. |
|---|---|
| Rebipelagar | 15,326 |
| Lactose broth | 10,182 |

In 72 samples the two methods agreed, that is the plate count was in approximate agreement with the reciprocal of the smallest quantity of the sample showing gas formation. In 25 samples the results differed by one dilution (the dilutions being decimal fractions of a cubic centimetre), in two samples by two dilutions, and in one sample by three dilutions. The agreement in the averages is very reasonable when the chance errors of distribution inherent to the tube method are considered, and the differences between individual samples can be shown to be well within the limits calculated by the theory of probabilities.

The errors connected with rebipelagar are caused (1) by the destruction of the characteristic colour of the B. coli colonies by the diffusion of amines or other alkaline substances through the medium and (2) by the development of red colonies by organisms not of the B. coli group. When a dilution of the sample is employed that prevents overcrowding of the colonies, the first error is usually avoided unless there is a large excess of alkali forming organisms present; this condition can be easily recognised because either a yellow area is produced concentrically from a colony, or, as is usually the case, the whole of the medium is yellow. The error due to organisms other than coliform bacteria is small and can be largely eliminated by experience. The characteristic forms produced by coliform organisms on the surface of the plate may either be a colony of deep red colour producing a haze in the surrounding medium, or one with a red centre surrounded by a yellowish or pinkish aureole of slimy consistency. The subsurface colonies are of the former variety but may not invariably produce the haze which is due to the diffusion of acid into the surrounding medium. The author, during the examination of several hundreds of coliform colonies from milk plated on rebipelagar, has only met with two organisms, one a coccus and the other a bacillus, that produced colonies resembling those typical of B. coli, but many organisms that ferment lactose with the production of acid may, especially after prolonged incubation, produce colonies that bear a superficial resemblance to those

described above. There is also a danger of mistaking pin point red colonies produced by acid-forming streptococci for those produced by attenuated B. coli and it will be found advisable to ignore all such colonies when examining the plates. By this procedure, only organisms in a fairly vigorous state are counted, and, although it is somewhat empirical in character, it produces results that are of greater sanitary significance. Of 271 red colonies fished from rebipelagar, the author found that 236 (87 per cent) were of the B. coli group so that even if all the red colonies are counted no serious errors will be introduced.

One difficulty in connection with the use of rebipelagar is the quality of the bile salt. Many brands of this salt are purchasable but very few are satisfactory. Sodium taurocholate, sodium glycocholate, and many brands of the commercial bile salt are too restrictive in their action on B. coli and if the amount is reduced to avoid this, the selective action is destroyed. With bile salt of satisfactory quality, vigorous B. coli will produce colonies 3 to 5 mm. in diameter in twenty-four hours at 37° C. and all brands that fail to do this should be rejected.

**Classification of B. Coli Type.** It has been indicated earlier in this chapter (page 136) that an attempt to regard one particular type of B. coli as having more sanitary significance than others has been a comparative failure. The present problem is not the definition of the properties of a distinct biotype such as B. coli communis or B. acidi lactici but the correlation of properties with the immediate previous environment. The fæcal types of B. coli can apparently be distinguished from those occurring on grain [11] by the hydrogen ion concentration produced in dextrose broth containing 0.5 per cent of dextrose, 1.0 per cent of peptone, and 0.2 per cent of acid potassium phosphate. This can best be determined by the methyl red reaction of Clark and Lubs [12] which Levine [13] has shown to be correlated with the Voges and Proskauer reaction. The precise sanitary significance of these so-called grain types

has yet to be determined but the present trend of opinion is towards the view that the methyl red negative, Voges and Proskauer positive types (grain types) are harmless saprophytes. The members of the B. coli group derived from human and bovine hosts can be partially distinguished by the usual reactions in sugar broths, the proteoclastic cleavage of gelatine, and the production of indol from peptone, but these reactions are not sufficiently specific for routine work although they have a limited application for research purposes.

**2. B. Enteritidis Sporogenes.** As the spores of B. enteritidis sporogenes are present in considerable quantities in manure and do not multiply in milk, the estimation of these would constitute an admirable test for original pollution if all other sources of these spores could be eliminated. The spores, however, may be derived from dirty vessels and in practice it is found that milk cans form a most fruitful source of these organisms. Milk cans, unless thoroughly sterilised with live steam, are very liable to contain large numbers of spores of various organisms as the treatment given, though usually sufficiently severe to kill the non-sporulating organisms, is not drastic enough to kill the spores. The usual temperature at which milk is pasteurised (143°–145° F.) is also not sufficiently high to kill the spores, so that the spore test is of considerable value in arriving at an opinion as to the bacteriological condition of pasteurised milk previous to pasteurisation. This test is, however, of much smaller value than the direct microscopical test previously described.

For the estimation of B. enteritidis sporogenes spores, various quantities of the milk are measured out into sterile test tubes, heated in a water bath at 80° C. for fifteen minutes, cooled, and incubated anærobically at 37° C. To obtain anærobic conditions the tubes may be placed in an air-tight jar containing alkaline pyrogallic, but satisfactory results may be obtained by covering the surface of the sample in each tube with paraffine; it is rather doubtful whether even this precaution is necessary, as the butter fat which rapidly rises and seals

the surface usually produces the necessary conditions. The method of Savage[10] is the most suitable with regard to the quantities of the sample to be tested. He suggests using ten tubes and placing 2 c.cms. in each tube, but this quantity may of course be varied in accordance with the nature of the sample. It is decidedly preferable to use a number of tubes containing small amounts of milk than only a few tubes containing larger amounts (*vide supra*). After two days incubation the tubes are examined for the "enteritidis change" which is indicated by a complete separation of the curd and the production of acid, the latter being easily detected by litmus solution. As other organisms, such as B. butyricus, give this reaction, it is not to be entirely relied upon, but these organisms are mainly non-pathogenic and may be differentiated by injecting 1 c.cm. of the whey subcutaneously into a guinea pig.

Using ten tubes containing 2 c.cms. each, the most probable number of spores present in 100 c.cms. of sample for each possible result is given in the Table LVIII, which is adapted from McCrady's results.[7]

TABLE LVIII

| Result. Positive Tubes. | Most Probable Number of Spores per 100 c.cms. |
|---|---|
| $\frac{0}{10}$ | 0 |
| $\frac{1}{10}$ | 5 |
| $\frac{2}{10}$ | 11 |
| $\frac{3}{10}$ | 17 |
| $\frac{4}{10}$ | 25 |
| $\frac{5}{10}$ | 34 |
| $\frac{6}{10}$ | 45 |
| $\frac{7}{10}$ | 60 |
| $\frac{8}{10}$ | 80 |
| $\frac{9}{10}$ | 114 |
| $\frac{10}{10}$ | Over 114 |

**3. Streptococci.** Cow manure contains 100,000 to 10,000,000,000 streptococci per gram, and the estimation of these

organisms in milk was long ago suggested as a means of determining manurial pollution, but, after considerable work had been done on the nature and significance of the streptococci usually found in milk this test fell into general desuetude. It was found that milk drawn under the best aseptic conditions contained streptococci which found milk an excellent nidus for reproduction and that it was practically impossible by simple tests to distinguish these organisms from those derived from manure. The examination of milk for Str. lacticus and Str. pyogenes will be discussed later, but it may be stated here that the identification of these organisms is far from being reliable and that their significance is still an open question.

For the *estimation* of streptococci, varying dilutions, as in the enrichment method for B. coli, are inoculated into neutral red dextrose broth tubes and incubated at 37° C. for two days. The sediment is then examined microscopically for long chains by means of a hanging drop preparation and all doubtful cases confirmed by stained smears. If desired, the streptococci may be isolated in pure culture, and the morphological and biochemical characteristics determined by spreading the diluted sediment over ordinary nutrient agar or whey agar and fishing off the isolated colonies after incubation. The properties of Str. bovis, Str. equinus and Str. fæcalis are given in Table LIX on page 155. The criticism made above with regard to the tube method for expressing a numeral value for B. coli applies equally to this method for estimating streptococci. As probably only excessive numbers of fæcal streptococci have any sanitary significance, the examination of a direct smear as in the Breed method for estimating the total number of bacteria or of a smear from a centrifugalised deposit, will give equally good results with less expenditure of time and labour.

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## CHAPTER VII

### PATHOGENIC ORGANISMS

**Streptococci.** Although the etiological relation of septic sore throat to infected milk has been noted on many occasions in Great Britain during the past thirty years, it is only during the past decade that any systematic investigations have been carried out and the bacteriology of this pathological condition developed. Probably the first bacteriological examination of any note was made in connection with the Angelsey outbreak of 1897 [1] when it was reported that Staphylococcus pyogenes and Streptococcus pyogenes were found in the milk but no B. diphtheriæ. Examination of the patients' throats gave similar results. Some of the most important contributions to the bacteriology of septic sore throat are those of Savage.[2] Of the 36 cases of mastitis investigated, 21, or 68 per cent were due to streptococci, 5, or 16 per cent to staphylococci, and the remainder to B. coli, B. tuberculosis and unclassified causes. On cultivation of the streptococci in the usual Gordon test media, it was found that a large percentage was of one type, called by Savage, Streptococcus mastiditis. This type tended to long chain formation and grew luxuriantly in broth forming a flocculent deposit above which the supernatant liquid remained clear. Lactose, dextrose, and saccharose were invariably fermented with the production of acid, and occasionally salacin, raffinose, and inulin. Mannite was never fermented. In milk acid was produced and a clot formed within three days; gelatin was not liquefied and no neutral red reaction was produced. It was non-pathogenic to mice. In 16 cases of sore throat Savage found the two chief varieties of streptococci to correspond to Andrewes and Holder's Str. anginosus and Str. pyo-

genes types with the former predominating (*vide* p. 155). The bovine type Str. mastiditis, and the human type Str. anginosus he was unable to distinguish either morphologically or biochemically, but a marked difference in virulence was found on animal injection. By auto inoculation on the tonsils Savage was unable to produce either local or general symptoms with Str. mastiditis even when massive doses were employed, and, in general, the organisms could only be recovered with difficulty even after such a short period as two to three days. The author has been unable to find any record of any tests being made by Savage as to the hæmolytic properties of the organisms isolated by him; this is of considerable importance, as hæmolysis is now generally regarded as characteristic of the pathogenic types Str. pyogenes and Str. anginosus.

Until 1911 septic sore throat seems to have been passed unrecognised in America, but the Boston epidemic in that year, with over 2000 cases, gave an impetus to the study of this disease, and since then it has proved to be one of the most fertile fields for research work. In the Boston epidemic, as in the later ones at Chicago, Baltimore, Concord (N. H.) and other places, the origin was traced to the milk supply and it was circumstantially established that the specific cause was a hæmolytic streptococcus of the pyogenes variety.

Krumwiede and Valentine[3] investigated an outbreak of septic sore throat on Long Island in 1914 and reported that it was caused by the transfer of pathogenic streptococci from a case of sore throat on a farm to one of the cows in the herd. An examination of the herd showed that five cows were giving milk containing a moderate number of streptococci from one or more quarters and that one of these gave physical evidence of mastitis. All these streptococci, however, were non-hæmolytic, but one other cow was found in which were moderate numbers of hæmolytic streptococci in two quarters and enormous numbers in a third quarter. The milk from this quarter was flocculent. These streptococci were morphologically and biochemically identical with those isolated from the throats of the

sufferers in the epidemic and from the probable original case. These organisms were of the Str. pyogenes type and fermented salicin but not raffinose or mannite.

Another link in the chain of evidence in favour of the streptococcal origin of these outbreaks, was founded by Jackson,[4] who showed that experimental arthritis could be produced in rabbits by the intravenous injection of hæmolytic streptococci. This is important on account of the frequency of joint infection as a sequel to septic sore throat as noted by many observers in the various epidemics.

Davis and Capps [5] endeavoured to produce an experimental infection of milk by smearing the uninjured teats of a cow with typical hæmolytic streptococci recently isolated from a case of streptococcal tonsilitis; this was unsuccessful, but on repeating the experiment after previously abrading the end of the teat near the meatus, an infection occurred and streptococci and leucocytes were found in abundance in the milk of the infected quarter. Similar results were produced by injecting the culture into the udder.

In view of the strong evidence that milk-borne streptococci were causative agents of septic sore throat it became imperative that a study should be made of the streptococci which are invariably found in milk, even though produced under the best conditions, in order to ascertain if there were any relation between these facts. Heinemann [6] has shown that Str. lacticus occurs constantly in milk and that the morphological and biochemical characteristics of this organism on ordinary media are identical with those of Str. pyogenes. Later [7] he found that by repeated passage through rabbits, he was able to exalt the virulence of Str. lacticus to such an extent that comparatively small doses were fatal. The lesions produced were very similar to those produced in human beings by Str. pyogenes. Müller [8] found that milk streptococci and pathogenic streptococci showed no material difference in their agglutination and hæmolytic properties but differed widely in the rapidity with which they coagulated milk. Heinemann in 1915 [9] reported

the results of further experiments on the pathogenicity of Str. lacticus and these in general confirm his earlier work. Two strains, one only of which was hæmolytic, but both capable of fermenting a variety of the usual test substances, were exalted in virulence by animal passage, and it is important to note that the fermentative capacity gradually decreased until finally one strain fermented only dextrose, and the other dextrose and saccharose. The non-hæmolytic strain became hæmolytic and both showed an increased tendency to chain formation. From these results Heinemann suggests that the determination of the fermentative ability of the streptococci might be of value in determining the previous environment of the organisms. If in contact with an animal lesion a low fermentative capacity would result whilst a high capacity would indicate a medium rich in carbohydrates.

Although the questions of the variability of streptococci in mastitis and the relation of mastitis to septic sore throat, are still far from being satisfactorily solved, it has been fairly definitely established that the great majority of the streptococci ordinarily found in milk are non-pathogenic and do not indicate a pathological condition of the udder. Str. lacticus, which may be found in almost every sample of milk, is used industrially in cheese manufacture and is also employed as a therapeutic agent. This streptococcus is typical of the group characterised by high fermentative capacity and low pathogenicity. The pathogenic streptococci, on the other hand, ferment but few of the Gordon test substances and produce low acidities in the media that are fermented; the morphological appearance is characterised by the picket fence (stalkett) formation but the chain may be either short or long; hæmolysis is marked.

**Examination for Streptococci.** Probably the most satisfactory method of examination for excessive numbers of streptococci resulting from mastitis, is the direct microscopical method of a smear prepared either by the Stewart-Sloan method described on page 126 or the Breed method described on page

129. In the microscopical examination, the streptococci having the typical form of Str. lacticus (elongated cocci, usually in pairs) should be ignored and a search made for the picket fence variety only. These, on staining with methylene blue, usually appear in chains with solidly stained portions at right angles to the longitudinal axis; capsules are usual but are not invariably found. Some observers attach more significance to the long-chain types, but in view of the numerous cases in which the short-chain types have been associated with pathological conditions, it would appear to be good policy to attach equal significance to both varieties. The property of chain formation is undoubtedly a variable one and is profoundly modified by the composition of the medium and general environment.

In the indirect method, the sample is diluted as in the examination for fæcal streptococci and the various dilutions seeded into dextrose broth. After incubation for forty-eight hours at 37° C., the cultures are examined for chain formation by making a smear or a hanging drop preparation; from the smallest quantity containing typical chains the approximate number of streptococci can be calculated. If desired, the broth cultures can be plated out on nutrient agar or gelatine, and the organisms isolated in pure culture. The quickest and most satisfactory method of examination for pathogenic streptococci is by plating on blood agar. Ruediger[41] as early as 1912 suggested the differentiation of Str. pyogenes from Str. lacticus by the hæmolytic properties of the former and since that date several workers have demonstrated that hæmolysis is a usual property of the pathogenic streptococci. All hæmolytic strains, however, are not pathogenic.

The best technique is to add various dilutions of the sample to 10 c.cms. of meat infusion agar containing 1 c.cm. of horse blood and then pour into Petri plates. These are incubated at 37° C. and examined after twenty-four and forty-eight hours for hæmolysis. Those colonies showing a clear, transparent, colourless zone are transferred to broth and finally inoculated in the usual Gordon test media, viz., dextrose, saccharose, raf-

finose, mannite, lactose, and salicin broths for determination of acidity, in milk for coagulation, and to blood agar plates for hæmolysis. A virulence test is also desirable, but in considering the results obtained due regard must be given to the dosage and method of inoculation. A quantity of broth that is sufficient to kill the test animal in three days when injected intravenously might not produce more than local symptoms when given subcutaneously, and similar conditions apply to the dosage. For guinea pigs 1 c.cm. of a forty-eight hour broth culture and for mice 0.5 c.cm. of a twenty-four hour culture have been found to give satisfactory results when injected into the peritoneal cavity.

The biochemical characteristics should be determined quantitatively by Winslow's method [39] if the best results are to be secured.

## Table LIX

BIOCHEMICAL CHARACTERS OF PRINCIPAL TYPES OF STREPTOCOCCI. (Broadhurst)

| Name of Variety. | Dextrose | Lactose. | Saccharose. | Raffinose. | Mannite. | Salicin. | Hæmolysis. | Gelatine Liquefaction. | Type Named by |
|---|---|---|---|---|---|---|---|---|---|
| Str. equinus... | X | O | X | O | O | X | − | − | Andrews and |
| Str. mitis..... | X | X | X | O | O | X | − | − | Horder |
| Str. pyogenes.. | X | X | X | O | O | X | + | − | " |
| Str. salicarius.. | X | X | X | ⊕ | O | O | | − | " |
| Str. anginosus.. | X | X | X | ⊕ | O | O | + | − | " |
| Str. gracilis.... | X | X | O | O | X | X | − | + | " |
| ? | X | X | O | O | X | X | − | − | " |
| Str. fæcalis.... | X | X | X | O | X | X | − | − | " |
| Str. versatilis.. | X | X | X | X | X | X | − | − | Broadhurst |
| Str. bovinus... | X | X | X | X | O | X | − | − | Winslow |

X indicates that test substance is fermented with production of acid and without gas formation.

⊕ indicates that test substance is occasionally fermented.

The fermentation and hæmolytic reactions of the best-known types of streptococci, excepting Str. lacticus, are shown in Table LIX.

### B. Diphtheriæ

Milk has, on several occasions, been proved to be a vehicle for B. diphtheriæ and responsible for epidemics of diphtheria, and it is consequently sometimes necessary for the bacteriologist to examine milk for this organism.

There is no satisfactory evidence that diphtheria organisms may invade the udder and so cause infection of the milk, but it is more than probable that milk has become accidentally infected from human sources and that the organisms have rapidly increased in number. Milk is not an ideal medium for the development of B. diphtheriæ but fairly rapid multiplication does occur until checked by the metabolic products of the acid producers.

The number of authentic cases in which B. diptheriæ has been isolated from milk are comparatively few. Bowhill,[10] in 1899, isolated diphtheria organisms from milk and prepared broth cultures that were fatal to guinea pigs in forty-eight hours. The same year Eyre [11] isolated a virulent diphtheritic bacillus from milk and, later, cases were reported by Klein,[12] Dean and Todd [13] and Marshall.[14]

For the isolation of the organisms, Bowhill directly inoculated Loeffler's blood serum with the sample. Eyre, and Dean and Todd concentrated the organisms by centrifugalising and afterwards streaked the sediment over a number of tubes of blood serum. The cream layer was treated in a similar manner. Characteristic colonies were fished and those morphologically resembling B. diphtheriæ isolated as pure cultures and tested for pathogenicity. Klein and Marshall used the animal inoculation method. The former inoculated two guinea pigs with one sample, one subcutaneously in the groin, and the other intraperitoneally. The latter pig remained well, but the former, on the fifth day, showed swollen inguinal glands sur-

rounded by soft œdematous tissue. On autopsy the subcutaneous tissue in the region of the seat of inoculation was œdematous and streaked with blood. The inguinal glands were enlarged, firm, and deeply congested. Film preparations from the juice of the incised gland showed numerous diphtheritic organisms. A pure culture was obtained which was proved to be B. diphtheriæ by the virulence test and also by the antitoxin test.

For the *examination* of milk for B. diphtheriæ, the serum method undoubtedly offers the best chance of obtaining a positive result. 50 c.cms. of sample are centrifugalised at 2000 revolutions per minute for twenty minutes and the cream layer removed to a sterile dish The milk layer is withdrawn by means of a suction pump connected to a fine bore glass tube until only 1–2 c.cms. remain. The sediment, and cream layer, are used for inoculating either blood serum plates or tubes. If tubes are used, one loopful is employed for smearing the surface of a number of tubes in succession so that at least one tube will be obtained in which the colonies are well isolated. In this manner a total of from 40 to 50 tubes is used for one sample and examined after sixteen or eighteen hours incubation at 37° C. From the tubes containing well-isolated colonies, subcultures are made of all colonies in any way resembling B. diphtheriæ and examined as to their morphological characteristics and biochemical properties. B. diphtheriæ is usually found in fresh serum preparations as a slender rod about $3\mu$ in length and exhibiting well-defined polar granules when stained with Loeffler's methylene blue or Ponder's stain (see appendix). The club-shaped bacillus is sometimes found, and also beaded and barred varieties but the bipolar type (type c, Westbrook classification) is the most typical. B. diphtheriæ does not liquefy gelatine, is Gram positive, and ferments dextrose, lævulose, galactose, arabinose, and maltose without formation of gas but not saccharose and mannite. Older cultures sometimes produce acid in lactose and glycerine. The bacillus is non-motile and does not form spores.

The organisms that pass the morphological and biochemical tests must be tested for virulence to guinea pigs. Two pigs are used, one for a subcutaneous or intra-peritonial injection of the twenty-four hour broth culture alone (1 c.cm.) and the other for a mixture of the culture with 1 c.cm. of a diphtheritic antitoxin of high titre. The unprotected pig usually dies within thirty-six hours, and almost invariably within forty-eight hours, if the culture is one of typical B. diphtheriæ. The protected animal should show no definite symptoms and remain alive.

**Diphtheroid Bacilli.** On many occasions bacilli have been found in milk having the characteristic granular staining properties of some forms of B. diphtheriæ but sharply differentiated from this organism by the absence of virulence. Bergey [15] investigated a number of these organisms which were apparently identical with B. diphtheriæ, and divided them into three groups according to their biochemical properties. Two groups showed fermentative activity markedly different to the diphtheritic group and that of the third was identical but non-pathogenic. Savage [16] investigated a number of the diphtheroid organisms found in milk sediments. These were apparently identical and closely resembled B. diphtheriæ in staining properties and microscopical appearance except for an absence of blue granules in preparations stained with Neisser's stain. The bacilli were Gram positive, non-motile, and developed on nutrient agar as small, discrete, translucent colonies. On serum they were slightly coloured and such organisms did not give the typical microscopical appearance found with the growths on agar. Litmus milk was unaffected and, except for a trace of acid in lactose, neither gas nor acid was produced in the usual test media. They were non-pathogenic to mice. Klein [17] found a bacillus in milk which he called B. diphtheroides. This organism differed morphologically from B. diphtheriæ, Hoffmann's bacillus, and the xerosis group. No growth was observed on gelatine at 21° C. or on agar at temperatures less than 25° C. On agar at 37° C. the growth was slow and no colonies appeared until the third day when they devel-

oped as small grey dots. Milk was coagulated at 37° C. with acid formation and a separation of the milk constituents into a cream layer at the top, curd at the bottom, and whey in between. On blood serum the colonies appeared on the third day as depressions due to liquefaction of the medium. On injection into guinea pigs, well-developed local abscesses appeared in one to two weeks. Intra-peritoneal injection produced abscesses on the omentum and on the pancreas or around the kidney. The author has, on several occasions, isolated bacilli from milk that resembled B. diphtheriæ, but the majority of these could be distinguished from the typical pathogenic variety by the size. The most usual type was about 5$\mu$ in length and slightly pointed at both ends; they retained the stain when treated by Gram's method and gave a typical barred appearance with Loeffler's methylene blue and Ponder's stain. On agar, and on blood serum, the organisms developed as small white opaque colonies. Gelatine was not liquefied. Dextrose, lactose, saccharose, mannite, and dulcite were not fermented and no visible change was produced in litmus milk. They were non-motile and did not form spores; broth cultures were non-pathogenic to guinea pigs when treated by the intra-peritoneal method.

No etiological connection has been discovered between these diphtheroid bacilli and any pathological condition and they must, therefore, be regarded as harmless saphrophytes that are of no importance or significance in public health work.

## B. Typhosus

There are on record several hundreds of epidemics of typhoid fever that are definitely attributed to milk as the immediate source of infection, but there is, so far as the author can ascertain, not a single authentic case recorded in which B. typhosus has been isolated from milk suspected of causing an epidemic. Typhoid infection of milk is of external origin and whether it is due to a carrier, or to a person having the dis-

ease, or water, it is almost invariably intermittent or transitory with the consequence that by the time an outbreak has occurred and can be traced to the milk supply it is almost hopeless to expect to isolate the infecting organism. This, however, should not deter those responsible for the investigation of such cases from attempting the isolation of B. typhosus.

**Isolation of B. Typhosus.** Jackson and Melia[18] recommend inoculating the sample into lactose bile and incubating at 37° C. The cultures are to be transplanted in varying dilutions into Hesse agar and examined after twenty-four hours at 37° C. On this medium B. coli forms small succinct colonies; B. typhosus is most characteristic on plates containing but few colonies; colonies of a large size are then formed, often several centimetres in diameter, and consisting of a broad translucent or scarcely turbid zone between a white opaque centre or nucleus and the perfectly circular narrow white edge. Tonney et al.[19] found that lactose bile is inhibitory to B. typhosus as well as to the colon group of organisms and this is confirmed by the author's experience.

The following method, which is an adaptation of Browning and Thornton's method[40] for the isolation of typhoid bacilli from fæces, can be recommended for the isolation of B. typhosus from milk. Centrifugalise 50 c.cms. of the sample for twenty minutes at 2000 to 2500 revolutions per minute. Remove the cream layer to a sterile tube and place it in a water bath at 37° to 40° C. Draw off the skim milk by means of a fine glass tube attached to a suction pump until about 3 c.cms. remain. After thoroughly distributing the sediment throughout the liquid it is inoculated into three brilliant green peptone tubes, one cubic centimetre being placed in each tube. The molten cream layer should be similarly treated as a proportion of the organisms may be trapped by the rising fat globules during the centrifugalising process. The brilliant green medium is prepared by steaming a 2 per cent peptone solution, containing 0.5 per cent of sodium chloride, for forty-five minutes and filtering after making the reaction slightly alkaline to litmus.

The medium is sterilised under pressure either in bulk or in 10 c.cm. quantities in tubes. The brilliant green (Hochst) is kept as a stock 1 per cent solution which is made into a 1 in 10,000 solution just before use by diluting 0.1 c.cm. to 10 c.cms. Before inoculating the 10 c.cms. of peptone saline medium with the suspected material, 0.5 c.cm. of the 1 in 10,000 brilliant green solution is added. The tubes are incubated at 37° C. for twenty to twenty-four hours and then plated out on neutral red bile salt agar or Endo's medium, preferably the former. The colourless characteristic colonies are fished and put through the usual agglutination and biochemical tests. Using this method, the author has been able to isolate B. typhosus from the sediment of milk to which had been added 23 typhoid bacilli per 100 c.cms.

**Paratyphoid-enteritidis or Gaertner Group.** The organisms of this group may be isolated by the same method as is given above for B. typhosus or, if no examination is required for B. typhosus, the sediment and cream may be inoculated into meat peptone dextrose broth (neutral to phenolphthalein) containing 0.15 c.cm. of a 1 per cent solution of brilliant green per 10 c.cms. of broth. (Tonney.[20]) This strength of brilliant green (1 in 6600) inhibits the growth of the Escherich and Eberth groups, and enables the Gaertner group to predominate the broth cultures. The broth cultures are subsequently plated out on neutral red lactose bile salt agar and the non-lactose fermenters worked out in the usual way.

**Morgan's Bacillus No. 1.** During the last few years the attention of sanitarians has been directed to the etiological relationship between milk supplies and epidemic summer diarrhœa. It has been evident for many years that artificial feeding of infants was a contributing factor but no definite cause was assigned for this phenomenon. Defective feeding has, no doubt, contributed to the excessive infantile mortality that occurs each summer, but there is a rapidly accumulating mass of evidence that the epidemic variety of summer diarrhœa is primarily or secondarily dependent upon the activity of micro-organisms. The substitution of a clean milk supply or the

pasteurisation of the old supply has, in many cases, led to an abatement of infantile diseases and this would indicate that an excessive number of bacteria of all kinds and not any particular group is responsible for the effects observed. (Park and Holt.[21])

Scholberg and Wallis[22] suggest that the prejudicial effect is due to physical and chemical changes produced by bacterial contamination. They found that the products of proteoclastic digestion appear in milk as the atmospheric temperature increased and that the albumoses and peptones so produced may be toxic to infants.

Morgan and Ledingham,[23] in 1909, made an investigation of the bacteriology of summer diarrhœa and concluded that a non-lactose fermenting, non-liquefying organism which they isolated and which is now usually known as Morgan's Number 1 Bacillus, bore a close relationship to the disease.

Lewis,[24] Ross,[25] O'Brien[26] and Orr,[27] made numerous examinations of the fæces of infants and, although they found that the non-gelatine liquefying, non-lactose fermenters were abnormally prevalent in the cases of diarrhœa, they could not establish any definite causal relationship. In 1911, Lewis[28] and Alexander[29] made further observations on this group and showed that Morgan's No. 1 Bacillus was conspicuously frequent in the fæces of infants having epidemic diarrhœa. In the same year Graham Smith[30] found that the non-gelatine liquefying non-lactose fermenters were especially prevalent in flies during the seasonal prevalence of diarrhœa and that Morgan's No. 1 Bacillus, whilst rarely present in flies from houses not containing diarrhœal cases, was frequently found in houses associated with this disease.

Lewis[31] pointed out the importance of applying the agglutination test to the various organisms which gave the usual fermentation reactions for Morgan's No. 1 Bacillus.

The etiological relationship of Morgan's No. 1 Bacillus to epidemic summer diarrhœa is not yet fully established, but the evidence in favour of this hypothesis is undoubtedly strong and points to the infection of the milk supply in the home by flies.

## Table LX

### BIOCHEMICAL CHARACTERS OF COLI-TYPHOID GROUP OF ORGANISMS

| | Motility. | Gelatine. | Dextrose. | Lævulose. | Galactose. | Maltose. | Lactose. | Saccharose | Raffinose. | Dextrin. | Inulin. | Salicin. | Glycerine. | Dulcite. | Mannite. | Sorbite. | Indol. | Litmus Milk. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | Early. | Late. |
| *The Escherich Group* | | | | | | | | | | | | | | | | | | | |
| B. coli communis | + | − | + | + | + | + | + | − | − | + | − | · | + | + | + | + | + | Acid | Acid |
| B. coli communior | + | − | + | + | + | + | + | + | + | + | − | · | + | + | + | + | + | Acid | Acid |
| B. lactis aerogenes | + | − | + | + | + | + | + | + | + | + | − | · | + | + | + | + | + | Acid | Acid |
| B. acidi lactici | ± | − | + | + | + | + | + | · | · | · | − | · | − | − | + | + | ± | Acid | Acid |
| B. cloaceæ | + | + | + | + | + | + | + | + | + | + | − | · | + | − | + | + | + | Acid | Acid |
| *The Gaertner Group:* | | | | | | | | | | | | | | | | | | | |
| B. enteritidis | + | − | + | · | + | + | − | − | − | − | − | − | − | + | + | + | − | .... | Alk. |
| B. paratyphosus. A | + | − | + | + | + | + | − | · | · | × | − | × | − | + | + | + | − | Acid | |
| B. paratyphosus. B | + | − | + | + | + | + | − | − | − | × | − | · | − | + | + | + | − | Acid | Alk. |
| B. choleræ suum | + | − | + | + | + | + | − | − | − | · | − | · | − | · | − | + | ± | Acid | Alk. |
| B. suipestifer | + | − | + | + | + | + | − | · | · | · | · | − | + | · | + | · | − | Acid | Alk. |
| Morgan's No. 1 | + | − | + | + | + | − | − | − | − | − | · | − | − | − | − | + | + | .... | Alk. |
| *The Ebert's group:* | | | | | | | | | | | | | | | | | | | |
| B. typhosus | + | − | × | × | × | × | − | − | − | × | − | − | − | − | × | × | − | Acid | Acid |
| B. dysenteriæ (Shiga) | − | − | × | × | × | − | − | − | − | · | − | − | − | − | − | − | − | Acid | Alk. |
| B. dysenteriæ (Flexner) | − | − | × | × | × | × | − | − | − | − | − | − | − | − | × | − | ± | Acid | Alk. |
| B. fæcalis alkaligenes | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | Alk. | Alk. |

Carbohydrate and broth media.

Legend: + = gas production. × = acid production without gas. − = no change.

**Examination.** Morgan's No. 1 Bacillus is very susceptible to the action of brilliant green and will not appear on the rebipelagar plates in the enrichment method for isolating the organisms of the Gaertner group. The best procedure is to inoculate the centrifugalised deposit from about 40–50 c.cms. of milk into a number of tubes of neutral red lactose bile salt agar and incubate for twenty-four to forty-eight hours after mixing and pouring into petri plates. All colourless colonies are fished into dextrose broth and those organisms producing acid and gas in this medium are afterwards tested in the usual media for biochemical reactions and also with a specific serum in low dilution. Morgan's No. 1 Bacillus invariably ferments dextrose and lævulose, and usually also arabinose and galactose, with the production of acid and gas. Mannite is also usually fermented but not saccharose, dulcite, maltose, dextrin, or salicin. Indol is produced in peptone water and milk becomes alkaline in about ten days. Gelatine is not liquefied.

## B. Tuberculosis

For the detection of B. tuberculosis in milk two processes have been employed: (*a*) the microscopical and (*b*) the inoculation method.

**Microscopical.** In very rare cases the presence of B. tuberculosis in milk may be demonstrated by the examination of stained films of the milk without previous concentration, but the percentage of positive results so obtained is so small as to render the process valueless for public health work. When the organisms are comparatively numerous they may be found in the deposit obtained by centrifugalising 50 to 100 c.cms. of milk at 2000 to 3000 revolutions per minute for thirty minutes. For the preparation of cover-slip films Delépine[32] recommends spreading small portions of the sediment over cover slips which, when dry, are placed in a covered capsule containing equal parts of absolute alcohol and ether for two hours. At the expiration of this period the capsule is placed in a dish con-

taining water at 35° to 40° C. The mixture of alcohol and ether boils at once and after ten to fifteen minutes the cover slips are removed and washed with absolute alcohol. The films are then stained with carbol-fuchsine and counterstained with methyline blue according to the Ziehl-Neelson method which is as follows:

(1) Stain in hot carbol-fuchsine for five to ten minutes, being careful to avoid over-heating.

(2) Decolourise by dipping in 25 per cent sulphuric acid.

(3) Wash in water.

(4) Wash in alcohol until no more stain is removed.

(5) Wash in water.

(6) Counterstain for one minute with methylene blue.

(7) Wash in water, dry, and mount.

Delépine found that when this method of preparation was carefully followed, very clear films were obtained and no difficulty was caused by other acid fast bacilli when sufficient attention was paid to the morphological characteristics of the organisms.

**Inoculation Method.** The inoculation method is the only one that can be relied upon for the detection of very small numbers of B. tuberculosis in milk, but the time required to obtain reliable results is not less than three weeks as compared with the few hours required for the completion of the microscopical method. It is good routine practice to make microscopical preparations of all sediments obtained by centrifugalisation and to inoculate those yielding negative or doubtful results.

To prepare the sediment, 100 c.cms. of milk are centrifugalised at 2000 to 3000 revolutions per minute for at least thirty minutes, and, after removing the cream layer with a sterile spatula or spoon, the separated milk is drawn off through a small bore glass tube attached to a suction pump until about 4 c.cms. of milk remain. This milk is thoroughly mixed with the deposit and subsequently used for the inoculation of two animals. If the milk is known to be "clean" the milk may

be reduced to 2 c.cms. and only one animal used for the deposit, the other being reserved for a portion of the cream layer.

On account of its sensitiveness to tuberculosis, the guinea pig is the most suitable animal for inoculation and the best results are obtained with animals weighing from 200 to 300 grams.

Two methods of inoculation are in general use: (*a*) subcutaneous injection at the inner side of the left hind leg and (*b*) intraperitoneal injection through the belly wall. Delépine prefers to inoculate at the inner aspect of the left leg at the level of the femoro-tibial articulation on account of the comparative results obtained by the uni-lateral development of the lesions. This, he found, was especially noticeable in the early stages with small amounts of infectious material and by noting the extent of the lesion development in two pigs killed after twenty-one and thirty-five days, a rough estimation of the degree of infectivity was procured. In the very early stages the lesions were limited to the subcutaneous tissue and the four groups of lymphatic glands (the popliteal, superficial inguinal, deep inguinal, and the sacro-lumbar) on the same side of the body as the seat of inoculation. Later the retro-hepatic gland and spleen were involved followed by the liver, lungs, bronchial suprascapular, and cervical glands on both sides of the body. Finally there was a more complete invasion of the lymphatic glands in front of the diaphragm on both sides of the body and an involvement of the superficial and deep inguinal and other glands behind the diaphragm on the right side of the body.

With the intra-peritoneal inoculation the lymphatic glands of the peritoneum and mysentery are first involved, followed by the liver and spleen. The cervical, bronchial, inguinal, and popliteal follow, but the lesion development is bilateral throughout.

In order to accelerate the development of the disease when the subcutaneous method is used, Block [33] suggested that the inguinal glands on the inoculation side should be slightly dam-

aged by squeezing them. This procedure reduces the resistance of the glands and enables an earlier diagnosis to be made. Dodd,[34] and Joannovico and Kapsammer[35] carefully studied this technique and found it entirely successful. They found that even doubtful cases could be diagnosed within fourteen days.

The microscopic appearance of the lesions is usually sufficient to enable a trained observer to make an accurate diagnosis, but in all doubtful cases cover slips preparations should be made and supplemented if necessary by histological sections. For cultures, nodules are squeezed between two sterile slides and the contents smeared over glycerine agar slopes. The cultures are incubated at 37° C.

For the differentiation of tubercular from other infections, Anderson[36] suggested the subcutaneous injection of 2 c.cms. of tuberculin. In a healthy animal a slight febrile reaction occurs and passes off in a few hours, but this quantity of tuberculin is sufficient to cause death in less than twenty-four hours in a guinea pig showing well developed tuberculosis. When the lesions are slight the animal will become sick but may not die. This method may be used as an addition to the usual autopsy but should not be substituted for it.

Even when the best technique is used, it is often found that the experimental animals may die from acute infections within a few days of inoculation. This is due to "dirty" milk and can be partially eliminated by the treatment of the sediment with 5 per cent antiformin for thirty minutes and finally washing with physiological saline. Eastwood and Griffith[37] found that 10 per cent antiformin slightly weakened the tubercle bacilli and that a 20 per cent solution almost destroyed them.

Death of the inoculated animals after ten days, from infections other than generalised tuberculosis, is largely due to improper attention to the housing conditions of the guinea pigs. These must be kept isolated in clean cages with not more than two animals to a cage and housed in well-ventilated rooms.

**Pseudo-tuberculosis.** Milk occasionally contains organisms capable of producing chronic lesions which partially simulate those of B. tuberculosis and to which the designation of pseudo-tuberculosis has been given. Delépine found that amongst these infections was one resembling chronic pyæmia, but in his opinion the resemblance is superficial and no experienced pathologist could mistake such lesions in the guinea pig for true tuberculous lesions; also that an experimenter with scanty pathological experience could not make a mistake if the organisms in the lesions are microscopically examined. The finding of the giant cells, characteristic of true tuberculosis, in histological sections would also clear up doubtful microscopic diagnoses.

In pigs that have been kept for five to six weeks the chronic lesions due to B. abortus may be found, but as this organism is not acid fast there is no difficulty in eliminating this possible source of error.

**Bovine and Human Types of B. Tuberculosis.** The difference in the cultural and other characteristics of these types is essentially relative rather than absolute and this fact must always be kept in mind when attempting to classify cultures of B. tuberculosis.

Eastwood and Griffith[38] classified cultures as dysgonic or eugonic according to the luxuriance of the growth on glycerinised agar and they found that the dysgonic type was usually of high virulence for rabbits and corresponded to the bovine type. The human type grew well on glycerine-agar but possessed much lower virulence for rabbits. The chief differences in the two types may be summarised as follows:

| | Bovine. | Human. |
|---|---|---|
| *Morphology.* | Only slight differences can be found, the bovine organisms being usually shorter, straighter, and thicker. | |
| *Cultural characteristics.* | | |
| *Glycerine-agar.* | Grows feebly and with development of discrete colonies. | Grows luxuriantly and usually without difficulty. Growth often wrinkled. |

| | |
|---|---|
| *Bovine serum.* Grows slowly and appears as a fine, filmy, non-pigmented growth after two to three weeks. | Grows fairly rapidly. |
| *Glycerine broth 2 per cent acid.* Acid reaction diminishes and may finally become alkaline. | Remains permanently acid. |
| *Pathogenicity.* | |
| Calves. Highly pathogenic. | Non-pathogenic. |
| Rabbits. Highly pathogenic. Subcutaneous inoculation with 10 m.gr. causes an acute generalised fatal tuberculosis. | Slightly pathogenic. The lesions are often localised in the lungs and kidneys or scattered. |

In the preparation of cultures from lesions for differentiation of type the primary ones should be made on Dorset's egg medium (see Appendix) and subcultivated to blood serum or glycerine-agar.

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# CHAPTER XIII

## CELLS, DIRT AND DEBRIS

**Cells.** For nearly a century it was recognised that cells or cell fragments were present in the secretion as formed in the alveoli, but it is only comparatively recently that any efforts were made to ascertain if any cells were present in the discharged milk. In 1897 Stokes and Wegefarth [1] directed attention to the presence of leucocytes in milk and, since then, considerable study has been given to this subject. These observers differentiated the leucocytes from the epithelial cells by the form of the nuclei but, unfortunately, designated the former as pus cells, a nomenclature that was perpetuated by many later writers. This designation is no longer accepted and the cells are regarded as constituents of normal milk. There is still some diversity of opinion regarding the nature of these cells, some experimenters, including Winkler, Hewlett, Villar, and Revis, holding that they are predominantly of epithelial origin, whilst others, amongst whom are Bergey, Doane, Miller, Breed, Ernst, and Savage, regard them mixtures of blood cells and epithelial cells.

Hewlett, Villar, and Revis [2] support the contention of Winkler and Michaelis that the cells in normal milk are chiefly young epithelial cells which have become detached. In a later paper they find that in the milk of healthy cows in full milk and which do not give a high cell count, the majority of the cells appear to be "large uninuclears" with a small admixture of other cells. At the beginning and end of lactation and when the cell count was high from other causes, whether physiological or pathological, the "multinuclears" predominated. Scannel [3] pointed out that epithelial cells are mononuclear and that,

although on dividing, they may appear as polymorphonuclears it is inconceivable that they should divide at such a rate as to produce 500,000 per c.cm. There are also certain histological characteristics that differentiate nucleated epithelial cells and mononuclear leucocytes.

The views of those who regard the cells found in milk as mixtures of blood and epithelial cells, which is the more generally accepted explanation, are well set forth in a recent book by Ernst [4] in which the histological characters of the cells are treated " in extenso."

According to Ernst the cells are of dual origin. (*a*) Epithelial cells derived from the tissue lining the ducts and from the secretory glands and,

(*b*) Leucocytes which have passed through the walls of the capillaries and lymphatics and finally obtained access to the gland secretion. This would appear to be normal process in all secretory glands. Under special stimulation, either from mechanical or pathological causes, the number and nature of the cells may undergo radical changes depending upon the nature and extent of the stimulation. This affords a rational explanation of the diversified cells found in milk and alterations in their relative proportions under varying conditions. A general description of the cells usually found in milk follows.

**Epithelial Cells.** (*a*) From compound epithelium: these are found as small platelets often folded in so many various ways that the original shape of the cell is entirely obscured. They are most numerous during the early period of the lactation and are due to the mechanical stimulation of the teats by milking.

(*b*) From the *milk cistern:* usually oval or rectangular in shape, frequently elongated to a point along the longitudinal axis and having an oval nucleus. In normal milk they are usually found singly but increased desquamation produced by stimulation may cause masses of cells to appear arranged like the petals of a flower round a common centre.

(*c*) From *secretory ducts and alveoli:* these vary in size according to the number of fat globules they contain (5 to 45 $\mu$) and

when very distended they are known as "foam cells." The nucleus is usually well marked when unmixed with fat and only surrounded with a narrow margin of protoplasm; the presence of fat produces the characteristic honeycombed appearance of the colostral bodies and such cells are only found in pathological conditions and at the beginning and end of the lactation period. Some observers report that these large cells may contain several nuclei, but Ernst never found more than one and suggested that the apparent multiplication of nuclei was due to mononuclear cells becoming superimposed.

**Blood Cells.** (*a*) *Red blood cells or erythrocytes* appear as biconcave discs or as thorn-apple shaped cells containing metachromatic granules.

(*b*) *Leucocytes.* These constitute a very considerable percentage of the total cells in normal physiological conditions and may entirely predominate in pathological ones. All varieties of leucocytes may be found but the usual frequency of occurrence is in the following order: polymorphonuclears, lymphocytes, large mononuclears, and transitionals.

The *polymorphonuclear leucocytes*, of which the majority are neutrophylic in their staining properties, are usually 7.5 to 10 $\mu$ in diameter and stain characteristically with methylene blue as a deeply stained lobed or polymorphonucleus surrounded by faintly coloured protoplasm. The *lymphocytes* are usually considerably smaller (5.7 $\mu$) than the "polymorphs" but vary very considerably in size. The nucleus is round and occupies practically the whole of the cell. *Mononuclear leucocytes* are much larger than the lymphocytes (usually 13–16 $\mu$ but may be 25 $\mu$ in diameter) and two to three times the size of erythrocytes. The nucleus is large and oval and is eccentrically situated in a relatively large amount of protoplasm. With methylene blue the nucleus stains moderately well and the cytoplasm contains fine amorphous particles which produce the appearance of ground glass. With Leishmann's stain the nucleus is ruby coloured and the cytoplasm blue but containing a few ruby granules. The *transitional cells* are about the size of

the large mononuclears. The nucleus shows varieties of transition between the indented mononuclear and the irregular polymorphonuclear cell. As a rule, it is indented, crescentic in shape, and not possessing the multiplication so characteristic of the polymorphonuclear leucocytes.

*Degenerated cells* of various kinds may also be present in milk. Cells may, under various influences, become partially or wholly disintegrated and the contents dispersed in fragments. The nucleus may split up and the chromatin spread through the plasma as dust or flakes. These flakes are often designated as " Nissen's Globules " and present the appearance of a darkly stained centre, with or without a lightly stained border. The albuminophores of Bab and Shulz which they describe as lymphocytes (15 to 20 $\mu$), containing fat and one to four proteid bodies, are regarded by Ernst as degenerated fat containing cells which have been attacked by macrocytes and then further degenerated until the nucleus is no longer visible.

**Estimation of Cells.** The first attempt to estimate the number of cells in milk was that of Stokes and Wegefarth in 1897 [1] and consisted in the examination under an oil immersion lens of a stained film prepared from the sediment obtained by centrifugal action. This method was adopted with but slight modifications by Bergey, Stewart and Slack.

Doane and Buckley in 1905 [5] devised what is known as the " volumetric method " in which a counting cell, such as is commonly used in the estimation of cells in blood, was used for the enumeration of the cells in the centrifugalised deposit from 10 c.cms. of milk. Russell and Hoffmann [6] compared the " smeared sediment " and " volumetric " methods and found an average variation of 112 per cent in the former as against only 6 per cent in the latter. They found also [7] that a preliminary heating of the milk to 70° C. produced higher and more consistent results. The details of this method, as adopted by the Committee on Standard Methods of Bacterial Milk Analysis of the American Public Health Association [8] are as follows:

**Collection of Samples.** Samples for analysis should be

taken from the entire milking of the animal, as the strippings contain a somewhat larger number of cells than other portions of the milk. For the purpose of examination take 200 c.cms. in a stoppered bottle.

**Time Interval between Collection and Analysis.** To secure satisfactory results, milk must be examined in a sweet condition. Development of acidity tends to precipitate casein in the milk and thus obscure the examination of microscopic preparations. Samples received from a distance can be preserved for satisfactory microscopical examination by the addition of formalin at the time of collection—a proportion of 1 c.cm. to 250 c.cms. of milk. Formalin has been found the best preservative to use although it causes contraction of the cells to some extent.

PROCEDURE WITH REFERENCE TO PREPARATION OF SAMPLE

**1. Heating Sample.** To secure the complete sedimentation of the cellular elements in the milk, it is necessary to heat the same to a temperature which will break down the fat globule clusters, or lessen the ordinary creaming properties of the milk. Samples should be heated at 65° to 70° C. for not less than ten minutes, or from 80° to 85° where very short periods of exposure (one minute) are given. This treatment causes the more homogeneous distribution of the fat globules through the milk, and when the sample is then subjected to centrifugal force, the cell elements are not caught in the rising fat globules, but on account of their higher specific gravity are concentrated in the sediment by centrifugal force.

**2. Concentration of Cellular Elements.** After centrifugalisation the cream and the supernatant milk are removed, with the exception of the last ½ c.cm., by aspirating with an exhaust pump and wiping the walls of the tube with a cotton swab. After thoroughly mixing the sediment with a glass rod, enough of the emulsion is placed in an ordinary blood counter (Thoma-Zeiss pattern) to fill exactly the cell. The preparation is then allowed to stand for a minute or two to permit the cellular

elements to settle to the bottom of the cell while the few fat globules in the liquid rise to the surface. This method permits of the differentiation of the cells from the small fat globules in the liquid so that a distinct microscopic observation can be made.

**Examination of Material.** The preparation is examined in an unstained condition. The count is made with a 1-inch eyepiece and $\frac{1}{6}$-inch objective. Where the number of cell elements exceed 12 or 15 per microscopic field, one-fourth of the entire ruled area of the counter, equivalent to 100 of the smaller squares of the cell, is counted. Where the cell elements are less abundant, one-half of the entire area (two to four hundred squares) is examined. The average number of cells per smallest square is then obtained, which when multiplied by 200,000 gives the number of cells per cubic centimeter in the original milk: multiplication by four million gives the number of cells per cubic centimetre in the sediment examined. As the sediment represents the concentration of cells into one-twentieth of the original volume of milk taken (10 c.c. to one-half c.c.) this number should be divided by twenty to give the number of cells per cubic centimetre in the original milk.

**Expression of Results.** All results should be expressed in number of cells per cubic centimetre of the original milk, and, in order to avoid fictitious accuracy and yet to express the numerical results by a method consistent with the precision of the work, the rules given below should be followed:

Numbers of Cells per c.cm.

| | | | |
|---|---|---|---|
| From | 1,001 to | 10,000 recorded to the nearest | 100 |
| | 10,001 | 10,000 | 500 |
| | 50,001 | 100,000 | 1,000 |
| | 100,001 | 500,000 | 10,000 |
| | 500,001 | 1,000,000 | 50,000 |
| | 1,000,001 | 10,000,000 | 100,000 |

Savage, in 1905, independently worked out a volumetric method based upon the same principle as the Doane-Buckley

method but differing radically in technique. This was published in 1906.[9] The method of Savage is the better one of the volumetric methods, so full details will be given: 1 c.cm. of milk is placed in a tube having a capacity of 15 c.cms. and diluted with Toisson's solution (see Appendix) until the tube is almost filled. The tube used is of special shape having the lower end about one-quarter the diameter of the general body of the tube and accurately graduated at 1 c.cm. After well mixing the fluids, the tube is centrifugalised at 1800 revolutions per minute for ten minutes. After breaking up the cream with a clean rod the tube is whirled for a further five minutes. The supernatant liquid is removed through a fine tube by means of a vacuum pump until just 1 c.cm. remains. After distributing the cells as evenly as possible in the sediment, a sufficient quantity is placed in the cell of a Thoma-Zeiss or some other convenient form of hæmocytometer and the cells counted in a number of fields of vision. Savage recommends drawing out the microscope tube until an exact number of squares spans the field of vision and gives the following formula for calculating the number of cells per cubic m.m.

$$\text{cells per cubic m.m. of milk} = \frac{56{,}000y}{11d^2},$$

where $y$ = the average number of leucocytes per field of vision, $d$ = the number of squares which just spans the diameter. This approximation of $\frac{56{,}000}{11}$ is accurate to within 0.5 per cent. The cells in the ruled squares can also be counted and the result calculated as in ordinary blood work, but as these represent but a small proportion of the total area of the cell, errors due to unequal distribution of the cells would be proportionately greater.

Hewlett, Villar and Revis add 6 drops of formalin to 60–70 c.cms. of milk in order to break down aggregations of cells and to prevent the cells being entangled in the cream layer. The

heating of the diluted milk tubes to 70° in Savage's method before centrifugalising would possibly produce higher results.

In 1910 Prescott and Breed [10] suggested the examination of the milk directly by means of stained smears. They found the results obtained by this method to be very much higher than by the Doane-Buckley method and that they were also more consistent. This was due to the varying number of cells trapped by the rising fat globules. Breed afterwards developed the process given on p. 129 which is obviously as applicable to cell examination as to the enumeration of bacteria. As previously mentioned, the accuracy of this method depends upon the even distribution of the cells and, if this condition does not obtain, a very large number of fields must be examined in order to obtain a fair average. With a cell count over 500,000 per c.cm. the author has obtained good results with this method but for smaller counts the method of Savage is to be preferred on account of the factor for the conversion of the cells per field to cells per unit volume being so much smaller.

**Significance.** Despite the numerous investigations that have been made in Europe and America during the last seventeen years, the significance to be attached to presence of cells in milk is still surrounded with difficulties. It has already been pointed out that a large number of cells are to be expected in the secretion of such an active organ as the udder even under normal physiological conditions and that stimulus, whether mechanical or pathological, results in an increase in numbers. As might be anticipated under such conditions the difficulty lies in establishing what might fairly be regarded as the normal variation in the number of cells. Savage found variations ranging from 50,000 to 1,000,000 cells per c.cm. Russell and Hoffmann found counts as high as 1,800,000 in animals in which there was no history of clinical disease while 33 per cent of the samples contained over 500,000 cells per c.cm. Stone and Sprague,[11] using the Doane-Buckley method, examined two healthy cows during the whole milking period (1,167 samples) with the following results:

| Samples. | Cell Count. |
|---|---|
| 1.2 per cent | under 10,000 per c.cm. |
| 7.0 | 10,000 to 20,000 |
| 61.0 | 20,000 to 100,000 |
| 29.0 | 100,000 to 500,000 |
| 1.8 | over 500,000 |

Breed and Stidger,[12] using the direct method, found variations ranging from 5000 to 20,000,000 cells per c.cm. in milk whcih they regarded as normal. Breed [13] examined 122 cows which averaged 868,000 cells per cubic centimetre; fifty-nine gave counts under 500,000 per cubic centimetre, 36 between 500,000 and 1,000,000 per cubic centimetre, and 27 gave counts over 1,000,000 per cubic centimetre.

Hewlett et al.[2] found that a change of feed influenced the cell count. As regards physiological influences, Savage [14] found that the previous number of calves and the age of the cow had apparently little or no effect; just after calving the leucocytes are increased, but after this condition has subsided the period since parturition has no effect until secretion commences to diminish. The cells at this period often show very abnormal values though not invariably so (Breed). Regarding the relative proportion of cells in the fore milk and middle milk the evidence is inconclusive, but it is agreed that there is an increase in the number discharged in the strippings. There are marked daily variations in the number of cells discharged and equally large ones in the product of the four quarters of one cow, for which no adequate explanation has been offered. Pathological conditions may increase the cell content very materially. Savage [14] obtained cell counts as high as 368,000,-000 per cubic centimetre in cases of mastitis and in these conditions he also found that the relative proportions of the cells approximated to those found in pus. The increased count was particularly due to polymorphonuclear leucocytes which represented 75 to 80 per cent of total number of cells. Even after the clinical evidence of mastitis has disappeared the cell count may continue to be excessive for a considerable period. Some

workers have endeavoured to find a relation between the cell count and the number of streptococci and other bacteria but with no marked success. Milk stasis has been shown by many observers to have a profound effect on the cell count by markedly increasing the number of leucocytes.

Whilst it is impossible to formulate any rigid standard for individual cows the author believes that mixed milk containing over 1,000,000 cells per cubic centimetre as determined by the Savage or Breed methods should be regarded with suspicion and the supply at once investigated. An excessive cell count is not sufficient, *per se*, to warrant condemnation of a supply, but if other unsatisfactory conditions also exist, such as large numbers of streptococci, the public should be protected by the exclusion of the supply until the condition is abated.

The tentative working basis of 1,000,000 cells per cubic centimetre is not so low as to prevent the possibility of passing a sample of mixed milk from a herd containing one case of garget but is sufficiently so to provide a reasonable safeguard without being oppressive on the producer. As a routine method of milk examination, the cell count has little to commend it in the case of herd milk, but in the examination of individual cows it is often of great service.

**Dirt and Debris.** During the present century many attempts have been made to quantitatively determine the amount of dirt and debris in milk. Several methods have been used, but as there is no agreement as to what is to be regarded as dirt these have given results which, although comparable among themselves, bear no relation to each other.

The sediment from milk according to Delépine [15] consists of

(*a*) Cells derived from the udders.
(*b*) Hairs and cells from the milker, or cows or other farm animals.
(*c*) Wool, cotton or other fibres from strainers, etc.
(*d*) Vegetable and mineral matter derived either from food, dung or litter or from dirty utensils and wash water.
(*e*) Algæ, moulds, and bacteria from various sources.

As the cells and bacteria are separately determined, the

estimation of the sediment somewhat overlaps in that direction and its amount, "cæteris paribus," should bear some relation to the number of cells and bacteria.

The methods that have been proposed for the estimation of the sediment in milk may be divided into two main groups.

(1) Preparation of sediment by centrifugalisation.

(2) Preparation of sediment by filtration.

**Group 1.** One of the oldest methods of this type is that of *Houston* [16] who added 1 c.c. of formalin to 1 litre milk and allowed the mixture to stand in a long tube with a narrow lower graduated extremity closed by a glass tap. A primary reading was obtained after twenty-four hours by making a direct observation on the scale. The sediment was then flushed out into a small graduated tube and the volume made up to 10 c.cms. with slightly alkaline water (0.1 per cent $Na_2CO_3$). After centrifugalisation for two minutes, a further observation was made. This was termed the "secondary reading." On account of the large volume of milk required, this method has not been generally adopted.

**Delépine, Babcock, and Gerber** all adopted methods in which the milk was centrifugalised for a specified time and the volume of sediment read off directly on the graduated lower extremity of the tube. Conn modified the usual centrifugal method by washing the sediment with distilled water and, finally, collecting it in tared filter papers which were afterwards dried and weighed. To convert the dry weight to a moist weight a factor was necessary and this was found to average 7. This factor was somewhat variable and depended upon the nature of the debris. Revis [17] uses a tube having a capacity of approximately 70 c.cms.; to this is attached a small glass cup by means of a ground-glass joint. Inside the constricted lower portion of the larger tube a glass rod is ground in to form a plunger valve.

In the determination, the lower glass cap is fitted and 50 c.cms. of milk placed in the tube which is then whirled for five minutes at 2000 revolutions per minute. After inserting the

rod valve, the lower tube is detached, the contents rejected and, after reconnecting with the lower tube, 50 c.cms. of distilled water are added and the valve withdrawn. After stirring the sediment thoroughly with a platinum needle, the tube and contents are given a further five minutes in the centrifuge. The supernatant liquid is removed as before but prior to the final washing with distilled water, the sediment is treated with 1 c.cm. of Eau de Javelle (antiformin may be substituted) for the purpose of dissolving the leucocytes and epithelial cells. After the final washing, the valve is inserted, and the lower cap removed and dried in the water oven with its contents. From the weight so obtained the tare of the cap is deducted and a correction made for a blank determination on the materials used. The dirt may be used for a microscopical examination. According to Revis, the hypochlorite has no action on dirt constituents, but in view of the well-known action of chlorine on cellulose this statement must be accepted with reserve.

**Group 2.** The filtration methods included in this group are practically all based on the filtration of a given volume of milk through a disc of cotton wool followed by an inspection of the disc for visible dirt.

Tonney [18] suggested the use of a small disc of absorbent cotton in a Gooch crucible and operated with reduced pressure obtained from a water pump. This is a fairly satisfactory procedure for laboratory examinations but is usually precluded by an insufficiency of sample. This principle of filtration for the purpose of demonstrating visible dirt has led to the manufacture of many commercial types of apparatus which have been used in dairies and creameries, and by milk inspectors, with more or less success. The types now on the market are the Lorenz or Wisconsin, Stewart, and Gerber, which use gravity filtration, and the Lorenz improved and Wizard which employ pressure or suction. A detailed account of these has been given by Schroeder [19] of the Health Department of New York City, but as these are of but very limited utility in laboratory work they will not be discussed " in extenso " here.

**Significance of Sediment.** If no efforts were made by producers and dairymen to remove sediment from milk, the determination of the dirt and debris would be an invaluable guide to the care exercised in the production and handling of milk, but in view of the fact that strainers or slime separators are in almost universal use, the amount of sediment may bear no relation whatever to the general condition of the milk. It has been shown by many sanitarians that the suspended debris represents only a small proportion of the total dirt and if this solid debris is removed by filtration or separation the general physical appearance of the milk might be entirely fallacious. The use of cotton disc filters by sanitary inspectors has accomplished much in the last few years by demonstrating to vendors in an incontrovertible manner the dirtiness of their product, but no real progress will be affected thereby if the farmer increases the efficiency of his strainers instead of preventing the access of dirt. There is a possibility that sanitarians may defeat their own objects by the placing too much reliance on the disc test and failing to correlate it with the bacterial count and other tests. Such "prima facie" evidence of cleanliness may be nothing but a specious fallacy.

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# CHAPTER IX

## PASTEURISED OR HEATED MILK

In addition to the usual bacteriological tests it is occasionally advisable to examine pasteurised milk with a view to determining the nature of the heat treatment to which it has been subjected. Prolonged heating at temperatures exceeding 150° F. results in the destruction of the enzymes and the loss of albumin and soluble phosphates; the fat globules may also be so altered that they do not rise normally and so affect what is commercially known as the "cream line."

The effect of time and temperature, the two factors controlling the general effect, have been admirably expressed by Dr. North of New York, in a diagram which, with slight modifications to bring it into harmony with the author's results, is reproduced on page 187.

For the detection of overheated milk, several methods are available: (1) determination of the cream line, (2) enzyme reactions, and (3) estimation of the albumin.

**Cream Line.** Place 100 c.cms. of the sample in a creamometer or graduated cylinder and observe the percentage of cream obtained after standing for six hours at 60° F. If less than 2.5 per cent of cream rises for each 1 per cent of fat contained in the original milk, the presence of heated milk must be suspected. If less than 2.5 per cent of cream is found for each 1 per cent of fat, the sample may either be milk pasteurised at a temperature exceeding 150° F., or a mixture of sterilised and fresh milk.

**Enzymes.** The effect of heat on milk enzymes has been studied by many workers and the more important results are given in Table LXI.

## Table LXI

### EFFECT OF HEAT ON ENZYMES IN MILK

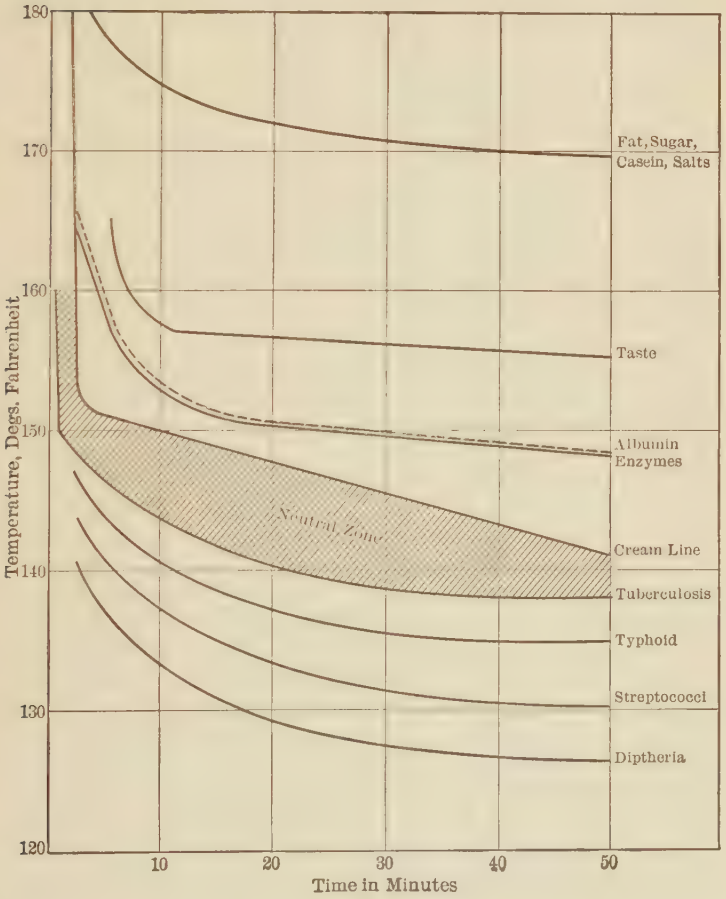
| Enzyme. | Authority. | Weakened | | Destroyed | |
|---|---|---|---|---|---|
| | | At Temp. ° C. | in Minutes. | At Temp. ° C. | In Minutes. |
| Galactase..... | Babcock and Russell | 65–70 | 10 | 76–80 | |
| | Von Freudenreich | 65–70 | 30 | 75–80 | |
| | Hippius | 65 | 30 | | |
| Amylase..... | Koning | ..... | ..... | 68 | 30 |
| | Hippius | ..... | ..... | 75–80 | |
| | Race | 68 | 30 | 83 | 30 |
| Lipase..... | Gillet | ..... | ..... | 65 | |
| Lactokinase... | Hougardy | ..... | ..... | 75 | 30 |
| Oxidases..... | Marfan | ..... | ..... | 79 | |
| | Hippius | ..... | ..... | 76 | |
| Peroxidases... | Wender | ..... | ..... | 83 | |
| | Schardinger | ..... | ..... | 80 | |
| | Ostertag | ..... | ..... | 80 | |
| | Lythgoe | 70 | 30 | 75 | 30 |
| | Race | 68 | 30 | 73 | 30 |
| | Numerous others. | 75 | ..... | 79–80 | |
| Catalase..... | Van Italie | ..... | ..... | 63 | 30 |
| | Wender | ..... | ..... | 80 | |
| Reductase.... | Jensen | ..... | ..... | over 70 | |
| | Lythgoe | 65 | 30 | 70 | 30 |
| | Race | 68 | 30 | 71 | 30 |

Although these results are slightly discordant, they all show that thirty minutes treatment at temperatures less than 65° C.

(149° F.) has no effect on the enzymes usually found in fresh milk.

The tests most easily applied are the hastened reductase

DIAGRAM NO. IV




reaction by means of Schardinger's reagent, and the peroxidase reaction with benzidine (page 91). The intensity of the

peroxidase reaction is inversely proportional to the intensity of the heat treatment and a similar indication is given if more than twenty to twenty-five minutes are required to discharge the blue colour in the reductase test.

The results obtained by the author on the effect of heat on the peroxidase and reductase tests are given in Tables LXII and LXIII.

### Table LXII

EFFECT OF HEAT ON PEROXIDASE TEST

| Duration of Heating in Minutes. | Benzidine Reaction after Heating to | | | | | |
|---|---|---|---|---|---|---|
| | 145° F. | 150° F. | 155° F. | 160° F. | 165° F. | 170° F. |
| 5 | + | + | + | + | + | + |
| 10 | + | + | + | + | + | + |
| 15 | + | + | + | + | + | Faint |
| 20 | + | + | + | + | Faint | Very faint |
| 25 | + | + | + | Faint | − | − |
| 30 | + | + | + | Very faint | − | − |

### Table LXIII

EFFECT OF HEAT ON REDUCTASE TEST

| Duration of Heating in | Time (Minutes) Required for Discharge of Colour after Heating to (Sample less blank). | | | | |
|---|---|---|---|---|---|
| | 145° F. | 150° F. | 155° F. | 160° F. | 170° F. |
| 5 | 0 | 1 | 1 | 3 | |
| 10 | 1 | 2 | 2 | 9 | Over 24 hr. |
| 15 | 2 | 3 | 3.5 | 30 | |
| 20 | 3 | 4 | 5 | 66 | Over 24 hr. |
| 25 | 3 | 4 | 6 | 204 | |
| 30 | 4 | 6 | 7 | Over 24 hr. | |

If milk has been treated with an excess of hydrogen peroxide or heated with a smaller quantity of this substance, the peroxidases are destroyed and a negative reaction is obtained with the usual reagents. Formaldehyde, in the quantities usually employed for milk preservation, has no apparent effect on the Schardinger test.

**Estimation of Albumin.** The estimation is most readily performed in the manner described on page 74.

Rupp [1] obtained the following results with heated milk.

| Milk Heated for Thirty Minutes at | Percentage of Albumin Precipitated. |
|---|---|
| 62.8° C. (145° F.) | Nil |
| 65.6° C. (150° F.) | 5.75 |
| 68.3° C. (155° F.) | 12.75 |
| 71.1° C. (160° F.) | 30.87 |

The rennin coagulation may also be used for the detection of sterilised milk or milk heated at temperatures exceeding 65° C. Rupp's results (*vide supra*) in this connection are given in Table LXIV.

## Table LXIV

Time required for rennin coagulation of raw and heated milk. Milk 200 c.cms.: rennin solution (0.15; 100 c.cms. water) 5 c.cms.

| Experiment. | Raw Milk. | | Milk Heated for Thirty Minutes at | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 55° C. 131° F. | | 60° C. 140° F. | | 65° C. 149° F. | | 70° C. 158° F. | | 75° C. 167° F. | |
| | Min. | Sec. | Min. | Sec. | Min. | Sec. | Min. | Sec. | Min. | Sec. | Min. | Sec. |
| 1 | 18 | 30 | 17 | 28 | 17 | 10 | 17 | 12 | | | | |
| | 19 | 08 | 16 | 56 | 16 | 53 | 17 | 12 | | | | |
| 2 | 19 | 34 | ..... | | ..... | | ..... | | 20 | 38 | 36 | 30 |
| | 19 | 23 | ..... | | ..... | | ..... | | 20 | 25 | 37 | 30 |

### Bacillus Abortus

Since 1897 when Bang and Stribald [2] isolated B. abortus as the causative agent of the infectious abortion in cattle, considerable study has been given to this organism in various parts of the world. McFadyean and Stockman [3] corroborated Bang's findings, but later work has resulted in the discovery of several allied forms with the consequence that B. abortus is now regarded as a species and not as a distinct biotype.

During the last decade several workers have found B. abortus in milk by the inoculation method and in some instances as many as 60 per cent of the samples gave positive results. The lesions produced by these samples were not usually sufficient to cause death.

Although the descriptions of B. abortus as given by various workers showed considerable variations, it remained for Evans [4] to classify the various forms and to indicate the relative frequency of certain varieties in normal udders. By plating milk on agar containing 10 per cent of bovine serum, Evans isolated B. abortus from 45 (23.4 per cent) of the 192 samples examined. These samples were obtained from 5 dairies. Thirty-three cultures exhibited a marked lipolytic action on milk fat and were, consequently, designated as B. abortus variety lipolyticus. Twelve cultures (variety *b*) differed from the pathogenic varieties in their ability to ferment the usual test substances, and morphology. The reactions of the varieties isolated by Evans are given in Table LXV, together with those of the typical pathogenic varieties for comparison.

B. abortus in young cultures shows the typical slender rod form but involution forms are often found in older ones and fœtal exudates often contain coccoid varieties. Ordinary aniline dyes may be used for staining purposes, carbol fuchsin followed by 1 per cent acetic acid, dilute carbol fuchsin, and Loeffler's methylene blue giving very satisfactory results. The organism is decolourised during Gram's method of staining. For cultural preparations agar containing 10 per cent of serum

may be used or an agar gelatine serum mixture (4 per cent gelatine, 6 per cent agar and 1 per cent serum) the serum in which is previously heated to 60° C. for one hour on 4 consecutive days to ensure sterility. This latter medium is very satisfactory for shake cultures. In carbohydrate media slightly variable results are recorded. Most workers report that neutral carbohydrate broths remain neutral or are rendered slightly alkaline, except for a few cultures which produce slight acidity in dextrose. Good and Corbett [5] report that B. abortus variety equinus showed an average of 2 per cent of gas in lactose in 93 cultures and no gas in 23. In saccharose 58 gave a little less than 2 per cent of gas and 28 were negative. Some cultures also produced marked quantities of gas in xylose, dextrose, arabinose, dulcite, sorbite, mannite, maltose, and raffinose. Duplicate and triplicate tests with lactose and saccharose gave varying results but Good and Corbett are convinced that the gas produced is the result of chemical action and not adventitious. From the results of the fermentation tests, these workers place the equinus variety in the Gaertner group of organisms. The great difference in fermentative ability between this variety and the other members of the B. abortus group would appear to warrant a change in the nomenclature of the equinus variety and its removal from the abortus group.

In guinea pigs, milk containing B. abortus often produces a nodular condition of the spleen and liver, the macroscopical appearance having a somewhat superficial resemblance to that produced by B. tuberculosis. In pregnant test animals, inoculation with cultures usually produces abortion in a few days but in some cases the action is much delayed and in others the gestation period may be quite normal.

### Acid-producing Organisms

Although the organisms found in milk capable of fermenting lactose with the production of acid include such widely differing groups as diplococci, staphylococci, streptococci, and bacilli,

TABLE

COMPARATIVE CHARACTERISTICS OF SEVERAL

| | B. abortus from Original Descriptions. | B. abortus from Pathogenic Sources. |
|---|---|---|
| Morphology. | Small rods, the largest as long as the tubercle bacilli. (Bang.) | Slender rods, 0.8 to 1.5 microns in length. |
| Reaction in dextrose, maltose, lactose, raffinose, mannite, and glycerine broth. | Alkaline broth is given an amphoteric or slightly acid reaction to Tournesol paper. (Nowak.) | Neutral broth is rendered slightly alkaline, except that a few cultures form a slight acidity in dextrose. |
| Decomposition of nitrogenous compounds. | | Nitrate, asparagin and urea are commonly decomposed. Gelatine is not liquified. |
| Action in litmus whole milk. | | Rendered slightly alkaline. |
| Growth in agar shake. | Growth in colonies is confined to a zone of from 10 to 15 mm. This zone lies about 5 mm. under surface of the agar. (Bang.) | Good growth on surface. Sometimes a growth throughout a zone of several mm. at the top. Rarely a diaphragm growth. |
| Growth on plain infusion agar slope. | Separate colonies resemble rose coloured droplets reflecting a greenish tinge. | Abundant compact growth chamois and cream buff in colour. |
| Growth in glycerine broth. | A poor growth. A fine sediment is thrown down made up of whitish grains. (Bang.) | Good growth which clouds the medium. |
| Effect of serum in the agar. | Growth is greatly favoured. (Bang.) | Abundant growth without serum. |

## LXV

VARIETIES OF BACILLUS ABORTUS. (After Evans)

| B. abortus Lipolyticus. | B. abortus Variety *b*. | B. abortus Variety *c*. |
|---|---|---|
| Slender rods, 0.8 to 1.5 microns in length. | Slender rods, 0.8 to 1.5 microns in length. | Slender rods, 0.8 to 1.5 microns in length. |
| No change. | Dextrose and maltose broths are rendered acid. No change in other broths. | Slightly alkaline. |
| Nitrate, and asparagin not decomposed; urea rarely. Gelatine not liquefied. | Nitrate, asparagin, and urea usually decomposed. Gelatine is not liquified. | Nitrate, asparagin and urea sometimes decomposed. Gelatine sometimes liquified. |
| Acid is developed in the cream layer. | Slightly alkaline in most cases. No change in others. | No change. |
| Colonies confined to a thin layer a few mm. beneath the surface. | Similar to those from pathogenic sources. Colonies sometimes scattered throughout the entire depth of agar. | Similar to the cultures from pathogenic sources. |
| A few cultures resemble those from pathogenic sources. The growth scanty in separate colonies. | Similar to the cultures from pathogenic sources. | Similar to cultures from pathogenic sources. |
| Scanty growth which does not cloud the medium. Sediment is made up of little granules. | Abundant growth. The medium is usually clouded, but sometimes the growth is precipitated, leaving a clear medium. | Similar to the growth of variety *b*. |
| Growth greatly favoured. | Abundant growth without serum. | Abundant growth without serum. |

it is sometimes desirable, as in the study of the effect of heat or chemical germicides upon the bacterial flora, to determine the relative proportion of this group to the total bacteria, without reference to the morphological characters of the individual members.

This division of the flora into groups on an acid-producing basis is necessarily an empirical one, but it is comparatively simple and has proved useful on many occasions.

The development of this method and its application to the examination of raw and pasteurised milk is largely due to Ayers and Johnson of the United States Department of Agriculture. In their earlier work they grouped the flora into acid forming, alkali forming, inert, and peptonising organisms according to their action on litmus lactose gelatine. This was effected by plating out the sample on this medium and counting the various groups after incubation for five days at 18° C. By this method it is often difficult to distinguish between the feeble acid formers, the feeble alkali formers, and the inert group, but fairly satisfactory results have been obtained with it in the author's laboratory [6] and it has the advantage of being much quicker and simpler than the later developments. The first modification made by Ayers [7] was an effort to obtain a more accurate count of the peptonising group by the elimination of spoilt plates caused by the spread of the gelatine liquefiers. A neutral lactose casein medium (see Appendix) was substituted for litmus lactose gelatine and the peptonisers differentiated by flooding the surface of the medium, after six days incubation at 30° C., with $\frac{N}{10}$ lactic acid. The colonies of peptonising organisms became white owing to the precipitation of casein by the acid. Ayers, in the same report (p. 227) also suggested the division of the flora into five groups according to the action on litmus milk. The colonies developing on lactose casein agar or infusion agar were fished into litmus milk tubes and incubated for fourteen days at 30° C. According to the appearance of the milk after this period the organisms were classified

as acid forming and coagulating, acid forming, inert, alkali forming, and peptonising. A comparison of the milk tube method and the litmus lactose gelatine plates was made by Ayers and Johnson [8] who obtained the following results as the averages of four samples.

| | Acid. | Alkali and Inert. | Peptonising. |
|---|---|---|---|
| After heating to 140° F.: | | | |
| Milk tubes | 71.5 | 22.8 | 5.7 |
| L. L. G. plates | 43.7 | 53.5 | 2.8 |
| After heating to 150° F.: | | | |
| Milk tubes | 84.6 | 10.5 | 4.9 |
| L. L. G. plates | 41.2 | 57.7 | 1.1 |

The milk tube method possesses the advantage of differentiating those organisms having feeble fermentative ability and also develops a larger proportion of peptonisers. The latter result may be partially due to the nature of the nitrogenous substance used for the test as it is exceedingly improbable that proteolysis proceeds at the same rate with all test substances.

**Aciduric Bacilli.** Among the acid-producing organisms, one sub-division, that of the aciduric or acidophylic bacteria, is especially worthy of further mention because it contains the commercially important B. bulgaricus. This organism has achieved considerable repute during the last few years as a therapeutic agent by reason of its influence on the flora of the intestinal canal and it has, consequently, become necessary to make bacteriological examinations of the tablets used for this purpose.

Although the aciduric bacilli grow luxuriantly in dextrose and lactose broth containing acetic or lactic acid they usually grow very sparingly or not at all on the usual laboratory media. They vary considerably in length (3 to 7 $\mu$) and occur singly or in chains or threads. They develop under both ærobic and anærobic conditions and, although typically Gram positive, old cul-

tures may be Gram negative. Spore formation is never observed and they ferment carbohydrates with the production of acid but do not form gas. Milk coagulation is produced by some members of the group and not by others.

For the isolation of this group there is no better method than that used by Heyman in 1898, viz., the use of a meat peptone broth containing 2 per cent dextrose and 0.3 per cent acetic acid. After incubation at 37° C. for forty-eight hours, a portion of the culture is seeded into another broth tube and the process repeated until only aciduric bacilli remain. For further isolation dextrose agar containing 1.5 per cent agar and 2 per cent dextrose without any adjustment of the acidity may be used. According to Rahe [9] the addition of 0.2 per cent of sodium oleate as recommended by Salge [10] is productive of good results. By this method Rahe (*vide supra*) investigated a number of the aciduric bacteria, and divided them into three groups according to their biochemical properties.

| Action on | Group. | | |
|---|---|---|---|
| | A. | B. | C. |
| Milk | Clot | Clot | No clot |
| Maltose | Not fermented | Fermented | Fermented |

Group A, which is the B. bulgaricus group, is characterised by a rapid clotting of milk and its usual inability to ferment carbohydrates other than lactose and dextrose.

Group B also clots milk but ferments maltose, saccharose, and lævulose in addition to lactose and dextrose, and usually also mannite and raffinose.

Group C does not clot milk and ferments maltose even more vigorously than group B. Saccharose and lævulose are fermented and usually raffinose, but mannite is not acted upon.

## The Fermentation Test in Milk Examination

This test is performed by incubating the sample in sterile vessels and observing the chemical and physical changes that take place.

The earliest experimental work in this connection was probably that of Walter, cantonal chemist at Soleure. This observer kept milk at 98° F., and stated that "milk, if good, will not curdle or undergo abnormal fermentation in ten to twelve hours." A special apparatus was devised for this purpose by Schaffer,[11] who recorded the amount of gas evolved in 100° F. from a definite volume of milk. He found that good milk formed no gas and remained fluid after twelve hours. This test was chiefly used in connection with the suitability of milk for cheese manufacture; milks that produced "heaving" were detected by this test.

The Wisconsin curd test[12] was also evolved for cheese manufacture and differs from the Swiss tests given above in the use of rennet for the production of a definite curd which is pressed and afterwards set aside for observation.

The Gerber fermentation test consists in incubating tubes of milk at 104° to 106° F. for six hours and then observing the odour, taste, and appearance for abnormal qualities. The heating is then continued for a second six-hour period and any abnormal coagulations, such as gas holes, are then noted. Gerber stated that coagulation in less than twelve hours is abnormal, and that milk that does not curdle in twenty-four hours to forty-eight hours is open to suspicion regarding preservatives.

According to Jensen,[13] the milk is heated to 30° to 35° C. for eight to twelve hours and examined; replaced for a further period and again examined. After the second period he found that the clean samples are sour and curdled and form a homogeneous coagulum without much separation of curd and gas formation. Frequently gas bubbles have split the coagulum and considerable fluid has separated. This change, he states,

does not necessarily signify that the milk was particularly rich in bacteria of putrefaction. If curdling is accompanied by an offensive odour or, if the coagulum is peptonised, the presence of putrefactive bacteria is inferred. He continues, "by boiling milk a short time and then incubating, only spore formers develop, and as these are not checked by the lactic bacteria, they increase rapidly and cause the milk to curdle by the action of ferments. Pasteurised milk does not sour, but no precipitate conclusions should be drawn from the results of this test."

Peter,[14] Dugelli,[15] and Klein [16] have used this test for milk examination and find that it gives the prevailing types of micro-organisms with a considerable degree of accuracy. A combination of the fermentation test with the methylene blue reduction test has been recommended by Lohnis and Schroeter,[17] and by Fred and Chappelean.[18]

In 1914 the author compared the results obtained by this test with the usual bacterial count on agar (forty-eight hours at blood heat) and the B. coli count in rebipelagar. The samples were transferred to sterile tubes plugged with absorbent cotton and incubated at 37° C. (98.5° F.) for 20–24 hours. 787 samples of ordinary raw milk, 98 samples of pasteurised milk, and 69 samples of nursery milk were examined in this way and the results recorded according to the classification of Dugelli (*vide supra*). This classification, together with the bacterial flora which Dugelli states is indicated by each type, is as follows:

### Types of Curd

#### *Type A*

**Liquid.** The sample does not show any marked change except perhaps a slight deposit on the bottom of the tube.

1. Completely liquid, sweet or sour taste.
2. Somewhat coagulated at the bottom or on the walls.
3. A slight ring of curd under the cream, but otherwise liquid and sour.

4. Completely liquid or with a slight separation of the solid components of the curd. Taste strongly acid or bitter acid.

*Type B*

**Gelatinous or Jelly-like.** The sample is more or less curdled and the casein is united into a gelatin-like mass without any marked separation of the curd.

1. A beautiful, smooth gelatinous mass without curd separation and a pure acid flavour.
2. Smooth but some gas bubbles and furrows.
3. Generally smooth, but with curd separation and marked by gas bubbles and furrows.
4. Generally smooth, with curd separation, but with numerous gas bubbles and furrows.

*Type C*

**Granular.** The milk curdles, but the curd, instead of being smooth consists of many small grains. Between the more or less fine curd grains, creamy cheese-like particles may be found.

1. Curd only partly granular and partly gelatin-like with little cheese separation.
2. Curd of fine granular structure and uniformly divided so that the curd looks white.
3. Curd shows a marked separation with mostly large grains.
4. Large granules and complete coagulation with a creamy deposit.

*Type D*

**Cheese Curd.** The casein is flocculent or in clumps, and is attached to the sides of the vessel. The curd is more or less completely separated from the whey.

1. Casein is a soft, united mass. The curd is greenish in colour and slightly acid.
2. Casein is a firm mass, curd green, and slightly acid.
3. Casein pulled apart and divided, a greenish white, strongly acid curd.

4. Casein entirely separated and attached to the sides of the tube. A white curd, strongly acid.

*Type E*

**Gaseous.** The tube is well marked with gas bubbles.

1. Cream filled with bubbles.
2. Cream and curd filled with bubbles.
3. Bubbles so numerous that the curd floats on the whey and forms a raised surface.
4. The gas development is so pronounced that the curd is forced upwards in the tube, often forcing out the stopper.

**Bacteria Flora,** as indicated by Fermentation Test. (*Dugelli.*)

*Type A*

Bacteria present in very small numbers. Cocci predominate with few lactic acid, coli and ærogenes organisms.

*Type B*

Lactic acid in great numbers, few if any coli and ærogenes organisms, some cocci and fluorescent bacteria. Gas formation indicates the presence of coli, ærogenes, or butyric organisms.

*Type C*

Lactic, coli, and ærogenes bacteria predominate with many cocci.

*Type D*

Lactic acid mixed with coli and ærogenes organisms.

*Type E*

Coli and ærogenes organisms abound if much gas is formed; also lactic bacteria, cocci and B. vulgatus.

The author's results showed that the type of fermentation was determined by a combination of factors which varied in different samples. The chief factors were the total and relative numbers of the various groups of organisms which constituted the bacterial flora.

When the total bacteria were very low the fermentation was usually of the A type, i.e., very little visible alteration occurred in the physical appearance of the sample, and a smooth acid flavour was produced. The acid producers were so few in numbers as to be unable to produce, under the incubator conditions, sufficient acid to coagulate the caseinogen. This is the distinguishing feature of type A. In types B, C, and D, there was a distinct coagulation, but the character varied in each group according to the organisms associated with the acid producers. The acid producers in each case produced their effect, and if the ratio of acid formers to gas formers were large, little or no evidence of gas formation was observed. As this ratio decreased furrows became evident and numerous gas bubbles were found enclosed in the curd, whilst in extreme cases the gas formation was so marked as to force the cream layer to the top of the tube. As any gas formed previous to the production of a firm curd would be lost without leaving any evidence, it follows that any gas observed must have been produced after coagulation and in a medium of increased acidity. To effect this the proportion of colon organisms must be considerable, as, otherwise, their development would be retarded by the metabolic products of the acid group. Very many samples, however, were observed to produce gas bubbles in the fermentation test, and yet contained originally less than one B. coli per cubic centimetre. In these cases either the small numbers of the B. coli must have increased very rapidly in proportion to the acid formers or be of an acid resisting type. At ordinary temperatures (50° to 60° F.), the colon content usually continued to increase until about 0.7 per cent of acidity, calculated as lactic acid, was produced.

The results also showed that the same type of fermentation

was produced by very widely differing B. coli contents, and it was, therefore, impossible to form a definite opinion regarding the B. coli content from the appearance of the fermentation test. The A type was almost invariably produced by milk low in B. coli, whilst D5 pointed to excessive contamination with this group, but with regard to the intermediate types, which the majority of market milks produce, no definite conclusions could be deduced. The same remarks apply regarding the relation of the total bacterial count to the type of fermentation, and, under these circumstances, it is difficult to attach much value to this test. Some observers have a high opinion of this test, because it is supposed to yield evidence as to bacterial flora and thus enable deductions to be made as to the conditions under which the milk was produced and its subsequent treatment, but the author's results do not substantiate this claim.

The conditions of the test, viz., incubation, at blood heat, are artificial, as milk is never, under ordinary circumstances, kept at this temperature, and it is not logically sound to assume that the biological and chemical changes are the same at different temperatures as a change of temperature always favours the growth of one or more groups in preference to others.

### Collection of Samples

All milk sold in bulk must be thoroughly mixed before samples are taken and every endeavour should be made to obtain milk in the same manner in which the vendor supplies the same to the consumer. The Committee of the American Public Health Association, appointed for the standardisation of bacteriological examination of milk, have recommended that bacteriological samples should be obtained from bulk milk by means of sterile pipettes, but this method samples milk which is in the possession of the vendor and ignores possible contamination in the vessel used for the transfer of such milk to the consumer. The author has observed numerous instances in

which this vessel has had very appreciable effects upon the bacterial count and the number of coliform bacteria. For the collection of combined chemical and bacteriological samples the author has used for several years rectangular, narrow-necked, six-ounce glass-stoppered bottles, 16 of which can be placed in a tray, 10 by $6\frac{1}{2}$ inches. This tray is surrounded with ice and water, and the whole contained in a water-tight galvanised-iron box $14\frac{1}{2}$ by $10\frac{1}{2}$ by 7 inches. In cold climates the cooling mixture can be dispensed with in winter and when there is any possibility of the milk freezing, wide-mouthed bottles should be used to prevent freezing of the sample and so blocking the neck of the bottle during the transfer of the sample. All milk retailed in bottle should be delivered to the laboratory in the original container unopened as the only other method of satisfactorily sampling such milk is to transfer the sample to a sterile bottle and then back to the original container, this being repeated several times. The sterile bottle necessary for the success of this method cannot usually be obtained so that this system should not be encouraged.

All samples should be labelled in such a way that there can be no possibility of doubt as to the identity of each sample and a complete record of the sampling data made immediately after the sample is taken. This should include name of vendor, date, time and place, temperature, character of container and name of collector. The temperature of milk in bulk is observed immediately after the sample has been taken whilst that of bottled milk should be obtained from a second bottle. A quickly reacting Fahrenheit thermometer is suitable for this purpose.

If the object of the examination of samples is to obtain figures representative of the total milk supply and from which averages can be calculated which are strictly comparative from month to month or from year to year, the collection of samples must be carried out as scientifically as possible and not in the usual haphazard fashion. The output of each vendor should be estimated and the number of samples varied in proportion

## Table LXVI

TOTAL BACTERIA AND B. COLI IN FARMERS' MILK YEAR ENDING OCTOBER 31, 1915. OTTAWA

| Month. | Number of Samples. | Variation in Bacterial Count per c.cm. Percentage of Samples Containing | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Under 10,000 | 10,001 to 50,000 | Under 50,000 | 50,001 to 100,000 | Under 100,000 | 100,001 to 500,000 | Under 500,000 | 500,001 to 1,000,000 | Under 1,000,000 | Over 1,000,000 |
| November... | 172 | 1.7 | 37.8 | 39.5 | 19.2 | 58.7 | 34.9 | 93.6 | 2.9 | 96.5 | 3.5 |
| December... | 137 | 2.9 | 45.3 | 48.2 | 22.6 | 70.8 | 22.6 | 93.4 | 5.9 | 99.3 | 0.7 |
| January..... | 122 | 2.5 | 36.1 | 38 6 | 21.3 | 59.9 | 36.9 | 96.8 | 1.6 | 98.4 | 1.6 |
| February.... | 139 | 12.2 | 34.5 | 46.7 | 25.2 | 71.9 | 25.2 | 97.1 | 2.9 | 100.0 | Nil |
| March..... | 175 | 3.4 | 44.6 | 48.0 | 19.4 | 67.4 | 28.0 | 95.4 | 4.0 | 99.4 | 0.6 |
| April..... | 114 | 3.5 | 21.1 | 24 6 | 18 4 | 43.0 | 40 3 | 83.3 | 13.2 | 96.5 | 3.5 |
| May..... | 136 | 3.7 | 32.3 | 36.0 | 16.2 | 52.2 | 36 0 | 88.2 | 8.1 | 96.3 | 3.7 |
| June..... | 151 | 4.0 | 15.2 | 19.2 | 13.2 | 32.4 | 44.4 | 76.8 | 15.9 | 92.7 | 7.3 |
| July..... | 155 | 3.9 | 12.9 | 16.8 | 20.6 | 37.4 | 36.8 | 74.2 | 17 4 | 91.6 | 8.4 |
| August..... | 159 | 1.3 | 8.8 | 10.1 | 13.2 | 23.3 | 55.3 | 78.6 | 15.1 | 93.7 | 6.3 |
| September... | 158 | 3.2 | 24.1 | 27.3 | 12.7 | 40.0 | 46.7 | 86.7 | 12.0 | 98.7 | 1.3 |
| October..... | 157 | 3.0 | 22 8 | 25.8 | 16 2 | 42 0 | 41 8 | 83 8 | 10.2 | 94.0 | 6.0 |
| Average..... | Total 1785 | 3.8 | 29.0 | 31.8 | 18.2 | 50 0 | 37.4 | 87.4 | 9.0 | 96.4 | 3.6 |

| Month. | Mean Bacterial Count per c.cm. | Mean Temp. of Atmosphere ° F. | Mean B. Coli Count per c.cm. | Variation in B. Coli per c.cm. Percentage of Samples Containing | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Under 10 | 11 to 100 | Under 100 | 101 to 1000 | Under 1000 | 1001 to 10,000 | Under 10,000 | 10,001 to 10,0000 | Under 100,000 |
| November... | 178,000 | 30.2 | 5,270 | 26.2 | 35.5 | 61.7 | 17.4 | 79.1 | 12.8 | 91.9 | 7.5 | 99.4 |
| December... | 144,000 | 16.8 | 1,698 | 32.2 | 43.8 | 76.0 | 13.1 | 89.1 | 7.3 | 96.4 | 3.6 | 100.0 |
| January..... | 133,000 | 15.3 | 819 | 47.6 | 30.3 | 77.9 | 12.3 | 90.2 | 7.3 | 97.5 | 2.5 | 100.0 |
| February.... | 103,000 | 19.8 | 443 | 61.8 | 22.4 | 84.2 | 9.4 | 93.6 | 5.0 | 98.6 | 1.4 | 100.0 |
| March..... | 118,000 | 26.4 | 707 | 51.4 | 34.3 | 85.7 | 7.5 | 93.2 | 5.7 | 98.9 | 1.1 | 100.0 |
| April..... | 216,000 | 49.0 | 2,309 | 30.7 | 32.5 | 63.2 | 18.4 | 81.6 | 9.6 | 91.2 | 8.8 | 100.0 |
| May..... | 209,000 | 51.6 | 5,034 | 27.9 | 25.7 | 53.6 | 21.3 | 77.9 | 13.2 | 91.1 | 8.2 | 99.3 |
| June..... | 354,000 | 63.6 | 10,000 | 22.5 | 12.6 | 35.1 | 27.8 | 62.9 | 15.9 | 78.8 | 18.6 | 97.4 |
| July..... | 372,000 | 68.0 | 12,300 | 8.3 | 20.0 | 28.3 | 24.1 | 52.4 | 23.4 | 75.8 | 21.4 | 97.2 |
| August..... | 358,000 | 64.5 | 14,300 | 3.2 | 11.9 | 15.1 | 23.3 | 38.4 | 32.7 | 71.1 | 26.4 | 97.5 |
| September... | 240,000 | 61.1 | 6,580 | 10.1 | 24 1 | 34 2 | 19 0 | 53 2 | 29.7 | 82 9 | 15 8 | 98.7 |
| October..... | 289,000 | 49.2 | 8,430 | 22.2 | 19.1 | 41.3 | 22.1 | 63.4 | 22.8 | 86.2 | 12.0 | 98.2 |
| Average..... | 226,000 | 42.9 | 5,657 | 26.7 | 26.3 | 55.0 | 17.9 | 72.9 | 15.5 | 88.4 | 10.6 | 99.0 |

# Table LXVII

## PASTEURISED MILK YEAR ENDING OCTOBER 31, 1915. OTTAWA

### Bacteriological

| Month. | Variation in Bacterial Count. Percentage of Samples containing | | | | | | |
|---|---|---|---|---|---|---|---|
| | Under 10,000 | 10,001 to 50,000 | Under 50,000 | 50,001 to 100,000 | Under 100,000 | 100,001 to 500,000 | Under 500,000 |
| November... | 41.2 | 47.0 | 88.2 | 5.9 | 94.1 | 5.9 | 100.00 |
| December... | 31.2 | 62.5 | 93.7 | 6.3 | 100.0 | Nil | |
| January..... | 53.8 | 46.2 | 100.0 | Nil | ..... | Nil | |
| February.... | 33.3 | 66.7 | 100.0 | Nil | ..... | Nil | |
| March..... | 43.8 | 56.2 | 100.0 | Nil | ..... | Nil | |
| April..... | 25.0 | 75.0 | 100.0 | Nil | ..... | Nil | |
| May..... | 81.8 | 18.2 | 100.0 | Nil | ..... | Nil | |
| June..... | 57.9 | 21.1 | 79.0 | 21.0 | ..... | Nil | |
| July..... | 57.1 | 38.1 | 95.2 | Nil | 95.2 | 4.8 | 100.0 |
| August..... | 50.0 | 50.0 | 100.0 | Nil | ..... | Nil | |
| September... | 31.6 | 63.2 | 94.8 | 5.2 | 100.0 | Nil | |
| October..... | 33.3 | 60.1 | 93.4 | 6.6 | 100.0 | Nil | |
| Average..... | 45.0 | 50.4 | 95.4 | 3.7 | 99.1 | 0.9 | 100.0 |

| Month | Mean Bacterial Count per c.cm. | Mean B. coli per c.cm. | Variation in B. coli per c.cm. Percentage of Samples | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Under 10 | 11 to 50 | Under 50 | 51 to 100 | Under 100 | Over 100 |
| November... | 25,000 | 3 | 88.3 | 11.7 | 100.0 | Nil | ..... | Nil |
| December... | 19,000 | 15 | 56.1 | 31.2 | 87.3 | 12.7 | 100.0 | Nil |
| January..... | 16,200 | 9 | 76.9 | 15.4 | 92.3 | 7.7 | 100.0 | Nil |
| February.... | 18,000 | 3 | 93.4 | 6.6 | 100.0 | Nil | ..... | Nil |
| March..... | 14,400 | 35 | 75.0 | 6.2 | 81.2 | Nil | 81.2 | 18.0 |
| April..... | 17,000 | 6 | 91.7 | 8.3 | 100.0 | Nil | ..... | Nil |
| May..... | 13,000 | 7 | 86.3 | 13.7 | 100.0 | Nil | ..... | Nil |
| June..... | 22,600 | 55 | 42.1 | 42.1 | 84.2 | 15.8 | 100.0 | Nil |
| July..... | 28,000 | 141 | 40.0 | 20.0 | 60.0 | 10.0 | 90.0 | 10.0 |
| August..... | 11,000 | 46 | 50.0 | 25.0 | 75.0 | 12.5 | 87.5 | 12.5 |
| September... | 33,000 | 896 | 47.3 | 31.6 | 78.9 | 10.4 | 89.3 | 10.7 |
| October..... | 18,700 | 6 | 86.7 | 13.3 | 100.0 | Nil | ..... | Nil |
| Average..... | 19,600 | 102 | 69.5 | 18.8 | 88.3 | 5.7 | 94.0 | 6.0 |

### Chemical

| Month. | Samples below Standard. Percentage of Samples. | | | Genuine Milks. Average Composition. | | | Number of Samples. |
|---|---|---|---|---|---|---|---|
| | Deficient in Fat. Solids. | Deficient Total Solids | Below 8.5 Per Cent Not-fat. Solids. | Fat. | Total Solids. | Solids Not-fat. | |
| November... | ..... | ..... | ..... | 4.03 | 13.02 | 8.99 | 17 |
| December... | ..... | ..... | ..... | 3.85 | 12.81 | 8.96 | 16 |
| January..... | ..... | ..... | ..... | 3.79 | 12.70 | 8.99 | 13 |
| February.... | ..... | ..... | ..... | 3.73 | 12.63 | 8.90 | 15 |
| March..... | ..... | ..... | ..... | 3.62 | 12.59 | 8.97 | 16 |
| April..... | ..... | ..... | ..... | 3.65 | 12.46 | 8.81 | 12 |
| May..... | ..... | ..... | ..... | 3.70 | 12.67 | 8.97 | 22 |
| June..... | ..... | ..... | ..... | 3.84 | 12.73 | 8.89 | 19 |
| July..... | ..... | ..... | ..... | 3.77 | 12.52 | 8.75 | 21 |
| August..... | ..... | ..... | 6.4 | 3.86 | 12.57 | 8.71 | 16 |
| Sept..... | ..... | ..... | ..... | 3.97 | 12.74 | 8.77 | 19 |
| October..... | ..... | ..... | ..... | 3.93 | 12.97 | 9.04 | 15 |
| Average..... | ..... | ..... | 0 5 | 3.81 | 12.72 | 8.91 | Total 201 |

to the output. When various grades of milk are offered for sale, the results should be separately recorded. The interval between sampling and examination should be as short as possible although no appreciable alteration occurs even in twenty-four hours if the samples are kept between 32° and 40° F.

**Recording Results.** The ordinary method of recording results by expressing the average total bacterial count or the average number of bacteria of some particular group of organisms, may give a result which does not represent the quality of the supply if the variations from the mean are large, or if the number of variants is comparatively small. The median would be more representative of the actual quality than the mean but a better plan is to express variations in the counts in the manner set forth in Tables LXVI and LXVII. The size of the groups in the scheme is quite arbitrary, but where milk is graded they should agree with the limits permitted in each particular grade.

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# APPENDIX

**Rebipelagar or Neutral Red Bile Salt Agar:**

| | |
|---|---|
| Agar | 20 grams |
| Peptone | 20 grams |
| Bile salt commercial | 5 grams |
| Water | 1000 c.cms. |

Heat the ingredients in a double pan or autoclave until completely dissolved; titrate with alkali and adjust the reaction to +1.0 per cent to phenolphthalein. Cool to 45° C., coagulate with egg albumen (5 grams dissolved in water), heat to boiling, adjust the weight and filter. Tube in convenient quantities, after adding 5 grams of lactose and 5 c.cms. of a 1.0 per cent solution of neutral red.

**Aesculin Bile Salt.** (Harrison and Vanderleck, Trans. Roy. Soc. of Canada, 1909, Sec. IV, 147.)

Dissolve in water 1.0 per cent of Witte's peptone, 0.25 per cent of bile salt, and 1.5 to 2.0 per cent of agar. Neutralise with alkali, coagulate with egg albumen and filter. Add 0.2 per cent of citrate of iron and 0.1 per cent of æsculin. This amount of citrate of iron should give a final acidity of +0.7 per cent and produces a slight fluorescence in the medium.

**Toissons's Solution:**

| | |
|---|---|
| Methyl violet | 0.025 gram |
| Sodium chloride | 1.0 gram |
| Sodium sulphate | 8.0 grams |
| Glycerine | 30 c.cms. |
| Distilled water | 160 c.cms. |

The solution should be freshly filtered.

**Ponder's Stain.** Kinyoun's modification.

| | |
|---|---|
| Toluidine blue | 0.1 gram |
| Azure I | 0.01 gram |
| Methylene blue | 0.01 gram |
| Glacial acetic acid | 1.0 c.cm. |
| 95 per cent alcohol | 5.0 c.cms. |
| Distilled water | 120 c.cms. |

The films should be stained for two minutes or more.

**Dorset's Egg Medium.** Take 12 fresh eggs, wash the shells with water and then with undiluted formalin; allow to dry. Break the eggs into a graduated cylinder and note the total volume. Add one part of sterile saline solution (0.85 per cent sodium chloride) to three parts of the mixed eggs. Pour into a sterile beaker or basin and whip with an egg whisk; filter through cheese cloth or muslin into a sterile flask and tube 10 c.cms. in the usual way. Inspissate at 75° C. for one hour in a sloping position and then add 0.5 c.cm. of sterile glycerine broth (physiological saline containing 6.0 per cent of glycerine) to each tube to prevent drying. Incubate at 37° C. for forty-eight hours and reject all contaminated tubes. Eyre recommends adding sufficient alcoholic basic fuchsin to produce a distinct colouration before the medium is tubed.

**Casein agar.** To 300 c.cms. of distilled water add 10 grams of casein (C. P. Hammersten) and 7 c.cms. of N. NaOH. Heat to boiling for several hours until thoroughly dissolved. Adjust the weight and bring the reaction to 0.2 per cent acid. The agar solution is prepared by dissolving 10 grams of agar in 500 c.cms. of water. Both solutions are filtered, mixed, tubed, and sterilised under pressure. The final reaction should be +0.1 per cent and, if the acidity is higher than this, a portion of the casein will be precipitated during sterilisation.

# Table LXVIII

## FOR CORRECTION OF SPECIFIC GRAVITY ACCORDING TO TEMPERATURE

| Temp. Deg. Fahr. | Degrees of Lactometer | | | | | | | | | | | | | | | | Temp. Deg. Fahr. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | |
| 45 | 19.0 | 19.9 | 20.9 | 21.9 | 22.9 | 23.8 | 24.8 | 25.8 | 26.7 | 27.7 | 28.6 | 29.5 | 30.4 | 31.4 | 32.3 | 33.2 | 45 |
| 46 | 19.0 | 20.0 | 21.0 | 22.0 | 22.9 | 23.9 | 24.9 | 25.9 | 26.8 | 27.8 | 28.7 | 29.6 | 30.5 | 31.4 | 32.4 | 33.3 | 46 |
| 47 | 19.1 | 20.0 | 21.0 | 22.0 | 23.0 | 24.0 | 24.9 | 25.9 | 26.8 | 27.8 | 28.7 | 29.6 | 30.5 | 31.5 | 32.5 | 33.4 | 47 |
| 48 | 19.1 | 20.1 | 21.1 | 22.1 | 23.1 | 24.0 | 25.0 | 26.0 | 26.9 | 27.9 | 28.8 | 29.7 | 30.6 | 31.6 | 32.6 | 33.5 | 48 |
| 49 | 19.2 | 20.2 | 21.2 | 22.2 | 23.2 | 24.1 | 25.1 | 26.1 | 27.0 | 28.0 | 28.9 | 29.8 | 30.7 | 31.7 | 32.7 | 33.6 | 49 |
| 50 | 19.2 | 20.2 | 21.2 | 22.2 | 23.2 | 24.1 | 25.1 | 26.1 | 27.0 | 28.0 | 29.0 | 29.9 | 30.9 | 31.8 | 32.8 | 33.7 | 50 |
| 51 | 19.3 | 20.3 | 21.3 | 22.3 | 23.3 | 24.2 | 25.2 | 26.2 | 27.1 | 28.1 | 29.1 | 30.0 | 31.0 | 31.9 | 32.9 | 33.8 | 51 |
| 52 | 19.4 | 20.3 | 21.3 | 22.3 | 23.3 | 24.3 | 25.2 | 26.2 | 27.2 | 28.2 | 29.1 | 30.1 | 31.1 | 32.0 | 33.0 | 33.9 | 52 |
| 53 | 19.4 | 20.4 | 21.4 | 22.4 | 23.4 | 24.4 | 25.3 | 26.3 | 27.3 | 28.3 | 29.2 | 30.2 | 31.2 | 32.1 | 33.1 | 34.0 | 53 |
| 54 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.4 | 26.4 | 27.4 | 28.4 | 29.3 | 30.3 | 31.3 | 32.3 | 33.3 | 34.2 | 54 |
| 55 | 19.6 | 20.6 | 21.6 | 22.6 | 23.6 | 24.6 | 25.5 | 26.5 | 27.5 | 28.5 | 29.4 | 30.4 | 31.4 | 32.4 | 33.4 | 34.3 | 55 |
| 56 | 19.7 | 20.7 | 21.7 | 22.7 | 23.6 | 24.6 | 25.6 | 26.6 | 27.6 | 28.6 | 29.6 | 30.5 | 31.5 | 32.5 | 33.5 | 34.4 | 56 |
| 57 | 19.8 | 20.8 | 21.8 | 22.8 | 23.7 | 24.7 | 25.7 | 26.7 | 27.7 | 28.7 | 29.7 | 30.6 | 31.6 | 32.6 | 33.6 | 34.5 | 57 |
| 58 | 19.9 | 20.9 | 21.9 | 22.8 | 23.8 | 24.8 | 25.8 | 26.8 | 27.8 | 28.8 | 29.8 | 30.8 | 31.7 | 32.7 | 33.7 | 34.7 | 58 |
| 59 | 19.9 | 20.9 | 21.9 | 22.9 | 23.9 | 24.9 | 25.9 | 26.9 | 27.9 | 28.9 | 29.9 | 30.9 | 31.9 | 32.9 | 33.9 | 34.8 | 59 |
| 60 | 20.0 | 21.0 | 22.0 | 23.0 | 24.0 | 25.0 | 26.0 | 27.0 | 28.0 | 29.0 | 30.0 | 31.0 | 32.0 | 33.0 | 34.0 | 35.0 | 60 |
| 61 | 20.1 | 21.1 | 22.1 | 23.1 | 24.1 | 25.1 | 26.1 | 27.1 | 28.1 | 29.1 | 30.1 | 31.1 | 32.1 | 33.1 | 34.1 | 35.1 | 61 |
| 62 | 20.2 | 21.2 | 22.2 | 23.2 | 24.2 | 25.2 | 26.2 | 27.3 | 28.3 | 29.3 | 30.3 | 31.3 | 32.3 | 33.3 | 34.3 | 35.3 | 62 |
| 63 | 20.2 | 21.3 | 22.3 | 23.3 | 24.3 | 25.3 | 26.3 | 27.4 | 28.4 | 29.4 | 30.4 | 31.4 | 32.5 | 33.5 | 34.5 | 35.5 | 63 |
| 64 | 20.3 | 21.4 | 22.4 | 23.4 | 24.4 | 25.4 | 26.5 | 27.5 | 28.5 | 29.5 | 30.5 | 31.5 | 32.6 | 33.6 | 34.6 | 35.6 | 64 |
| 65 | 20.4 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.6 | 27.6 | 28.6 | 29.6 | 30.7 | 31.7 | 32.7 | 33.8 | 34.8 | 35.8 | 65 |
| 66 | 20.5 | 21.6 | 22.6 | 23.6 | 24.6 | 25.6 | 26.7 | 27.7 | 28.7 | 29.8 | 30.8 | 31.8 | 32.9 | 33.9 | 34.9 | 35.9 | 66 |
| 67 | 20.6 | 21.7 | 22.7 | 23.7 | 24.7 | 25.7 | 26.8 | 27.8 | 28.8 | 29.9 | 30.9 | 32.0 | 33.0 | 34.0 | 35.0 | 36.1 | 67 |
| 68 | 20.7 | 21.8 | 22.8 | 23.8 | 24.9 | 25.9 | 27.0 | 28.0 | 29.0 | 30.1 | 31.1 | 32.1 | 33.2 | 34.2 | 35.2 | 36.2 | 68 |
| 69 | 20.9 | 22.0 | 23.0 | 24.0 | 25.0 | 26.0 | 27.1 | 28.1 | 29.1 | 30.2 | 31.2 | 32.2 | 33.3 | 34.3 | 35.3 | 36.3 | 69 |
| 70 | 21.0 | 22.1 | 23.1 | 24.1 | 25.1 | 26.1 | 27.2 | 28.2 | 29.2 | 30.3 | 31.3 | 32.4 | 33.4 | 34.5 | 35.5 | 36.5 | 70 |
| 71 | 21.1 | 22.2 | 23.2 | 24.2 | 25.2 | 26.2 | 27.3 | 28.3 | 29.4 | 30.4 | 31.5 | 32.5 | 33.6 | 34.6 | 35.6 | 36.7 | 71 |
| 72 | 21.2 | 22.3 | 23.3 | 24.3 | 25.3 | 26.4 | 27.4 | 28.4 | 29.5 | 30.5 | 31.6 | 32.6 | 33.7 | 34.7 | 35.8 | 36.8 | 72 |
| 73 | 21.3 | 22.4 | 23.4 | 24.4 | 25.5 | 26.5 | 27.5 | 28.6 | 29.7 | 30.7 | 31.8 | 32.8 | 33.9 | 34.9 | 36.0 | 37.0 | 73 |
| 74 | 21.5 | 22.5 | 23.5 | 24.6 | 25.6 | 26.6 | 27.7 | 28.7 | 29.8 | 30.9 | 31.9 | 33.0 | 34.1 | 35.1 | 36.2 | 37.2 | 74 |
| 75 | 21.6 | 22.6 | 23.7 | 24.7 | 25.7 | 26.8 | 27.8 | 28.9 | 29.9 | 31.0 | 32.1 | 33.2 | 34.3 | 35.3 | 36.4 | 37.4 | 75 |

## TABLE

## FOR CALCULATION OF TOTAL SOLIDS

According to Babcock.

Lactometer Reading

| Fat. | 26.0 | 26.5 | 27.0 | 27.5 | 28.0 | 28.5 | 29.0 | 29.5 | 30.0 | 30.5 | 31.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 6.50 | 6.62 | 6.75 | 6.87 | 7.00 | 7.12 | 7.25 | 7.37 | 7.50 | 7.62 | 7.75 |
| 0.1 | 6.62 | 6.74 | 6.87 | 6.99 | 7.12 | 7.24 | 7.37 | 7.49 | 7.62 | 7.74 | 7.87 |
| 0.2 | 6.74 | 6.86 | 6.99 | 7.11 | 7.24 | 7.36 | 7.49 | 7.61 | 7.74 | 7.86 | 7.99 |
| 0.3 | 6.86 | 6.98 | 7.11 | 7.23 | 7.36 | 7.48 | 7.61 | 7.73 | 7.86 | 7.98 | 8.11 |
| 0.4 | 6.98 | 7.10 | 7.23 | 7.35 | 7.48 | 7.60 | 7.73 | 7.85 | 7.98 | 8.10 | 8.23 |
| 0.5 | 7.10 | 7.22 | 7.35 | 7.47 | 7.60 | 7.72 | 7.85 | 7.97 | 8.10 | 8.22 | 8.35 |
| 0.6 | 7.22 | 7.34 | 7.47 | 7.59 | 7.72 | 7.84 | 7.97 | 8.09 | 8.22 | 8.34 | 8.47 |
| 0.7 | 7.34 | 7.46 | 7.59 | 7.71 | 7.84 | 7.96 | 8.09 | 8.21 | 8.34 | 8.46 | 8.59 |
| 0.8 | 7.46 | 7.58 | 7.71 | 7.83 | 7.96 | 8.08 | 8.21 | 8.33 | 8.46 | 8.58 | 8.71 |
| 0.9 | 7.58 | 7.70 | 7.83 | 7.95 | 8.08 | 8.20 | 8.33 | 8.45 | 8.58 | 8.70 | 8.83 |
| 1.0 | 7.70 | 7.82 | 7.95 | 8.07 | 8.20 | 8.32 | 8.45 | 8.57 | 8.70 | 8.82 | 8.95 |
| 1.1 | 7.82 | 7.94 | 8.07 | 8.19 | 8.32 | 8.44 | 8.57 | 8.69 | 8.82 | 8.94 | 9.07 |
| 1.2 | 7.94 | 8.06 | 8.19 | 8.31 | 8.44 | 8.56 | 8.69 | 8.81 | 8.94 | 9.06 | 9.19 |
| 1.3 | 8.06 | 8.18 | 8.31 | 8.43 | 8.56 | 8.68 | 8.81 | 8.93 | 9.06 | 9.18 | 9.31 |
| 1.4 | 8.18 | 8.30 | 8.43 | 8.55 | 8.68 | 8.80 | 8.93 | 9.05 | 9.18 | 9.30 | 9.43 |
| 1.5 | 8.30 | 8.42 | 8.55 | 8.67 | 8.80 | 8.92 | 9.05 | 9.17 | 9.30 | 9.42 | 9.55 |
| 1.6 | 8.42 | 8.54 | 8.67 | 8.79 | 8.92 | 9.04 | 9.17 | 9.29 | 9.42 | 9.54 | 9.67 |
| 1.7 | 8.54 | 8.66 | 8.79 | 8.91 | 9.04 | 9.16 | 9.29 | 9.41 | 9.54 | 9.66 | 9.79 |
| 1.8 | 8.66 | 8.78 | 8.91 | 9.03 | 9.16 | 9.28 | 9.41 | 9.53 | 9.66 | 9.78 | 9.91 |
| 1.9 | 8.78 | 8.90 | 9.03 | 9.15 | 9.28 | 9.40 | 9.53 | 9.65 | 9.78 | 9.90 | 10.03 |
| 2.0 | 8.90 | 9.02 | 9.15 | 9.27 | 9.40 | 9.52 | 9.65 | 9.77 | 9.90 | 10.02 | 10.15 |
| 2.1 | 9.02 | 9.14 | 9.27 | 9.39 | 9.52 | 9.64 | 9.77 | 9.89 | 10.02 | 10.14 | 10.27 |
| 2.2 | 9.14 | 9.26 | 9.39 | 9.51 | 9.64 | 9.76 | 9.89 | 10.01 | 10.14 | 10.26 | 10.39 |
| 2.3 | 9.26 | 9.38 | 9.51 | 9.63 | 9.76 | 9.88 | 10.01 | 10.13 | 10.26 | 10.38 | 10.51 |
| 2.4 | 9.38 | 9.50 | 9.63 | 9.75 | 9.88 | 10.00 | 10.13 | 10.25 | 10.38 | 10.50 | 10.63 |
| 2.5 | 9.50 | 9.62 | 9.75 | 9.87 | 10.00 | 10.12 | 10.25 | 10.37 | 10.50 | 10.62 | 10.75 |
| 2.6 | 9.62 | 9.74 | 9.87 | 9.99 | 10.12 | 10 24 | 10.37 | 10.49 | 10.62 | 10.74 | 10.87 |
| 2.7 | 9.74 | 9.86 | 9.99 | 10.11 | 10.24 | 10.36 | 10.49 | 10.61 | 10.74 | 10.86 | 10.99 |
| 2.8 | 9.86 | 9.98 | 10.11 | 10.23 | 10.36 | 10.48 | 10.61 | 10.73 | 10.86 | 10.98 | 11.11 |
| 2.9 | 9.98 | 10.10 | 10.23 | 10.35 | 10.48 | 10.60 | 10.73 | 10.85 | 10.98 | 11.10 | 11.23 |
| 3.0 | 10.10 | 10 22 | 10.35 | 10 47 | 10 60 | 10 72 | 10 85 | 10 97 | 11 10 | 11.23 | 11 36 |
| 3.1 | 10.22 | 10 34 | 10.47 | 10.59 | 10 72 | 10 84 | 10 97 | 11.09 | 11 22 | 11.35 | 11.48 |
| 3.2 | 10.34 | 10.46 | 10 59 | 10 71 | 10 84 | 10 96 | 11 09 | 11.21 | 11.34 | 11.47 | 11.60 |
| 3.3 | 10 46 | 10 58 | 10 71 | 10 83 | 10 96 | 11 09 | 11.21 | 11 34 | 11 46 | 11 59 | 11.72 |
| 3.4 | 10.58 | 10.70 | 10 83 | 10 96 | 11 09 | 11 21 | 11 34 | 11 46 | 11 58 | 11.71 | 11.84 |
| 3.5 | 10 70 | 10 82 | 10 95 | 11 09 | 11.21 | 11.33 | 11 46 | 11 58 | 11.70 | 11.83 | 11.96 |
| 3.6 | 10.82 | 10.95 | 11.08 | 11.20 | 11 33 | 11 45 | 11 58 | 11.70 | 11.82 | 11.95 | 12 08 |
| 3.7 | 10.94 | 11.07 | 11 20 | 11.32 | 11.45 | 11 57 | 11.70 | 11 82 | 11.94 | 12.07 | 12.20 |
| 3.8 | 11.06 | 11.19 | 11 32 | 11.44 | 11.57 | 11 69 | 11 82 | 11 94 | 12 06 | 12 19 | 12.32 |
| 3.9 | 11.18 | 11.31 | 11 44 | 11.56 | 11.69 | 11 81 | 11.94 | 12 06 | 12.18 | 12.31 | 12.44 |
| 4.0 | 11.30 | 11.43 | 11.56 | 11.68 | 11 81 | 11.93 | 12 06 | 12.18 | 12.31 | 13 43 | 12.56 |
| 4.1 | 11.42 | 11.55 | 11.68 | 11.80 | 11.93 | 12.05 | 12.18 | 12.30 | 12 43 | 12.55 | 12.68 |
| 4.2 | 11.54 | 11.67 | 11.80 | 11.92 | 12.05 | 12.17 | 12.30 | 12.42 | 12 55 | 12.67 | 12.80 |
| 4.3 | 11.66 | 11.79 | 11 92 | 12 04 | 12.17 | 12.29 | 12.42 | 12.54 | 12.67 | 12.79 | 12.92 |
| 4.4 | 11.78 | 11.91 | 12 04 | 12 16 | 12.29 | 12 41 | 12.54 | 12 66 | 12.79 | 12.91 | 13.04 |
| 4.5 | 11 90 | 12.03 | 12.16 | 12 28 | 12.41 | 12 53 | 12 66 | 12 78 | 12 91 | 13.03 | 13.16 |
| 4.6 | 12 03 | 12 15 | 12.28 | 12 40 | 12.53 | 12 65 | 12.78 | 12 90 | 13.03 | 13.15 | 13.28 |
| 4.7 | 12.15 | 12.27 | 12.40 | 12.52 | 12.65 | 12.77 | 12.90 | 13 02 | 13.15 | 13.27 | 13.40 |
| 4.8 | 12.27 | 12 39 | 12.52 | 12 64 | 12.77 | 12 89 | 13 02 | 13.14 | 13 27 | 13.39 | 13.52 |
| 4.9 | 12.39 | 12.51 | 12.64 | 12 76 | 12.89 | 13 01 | 13.14 | 13.26 | 13.39 | 13.51 | 13.64 |
| 5.0 | 12.51 | 12.63 | 12.76 | 12.88 | 13.01 | 13.13 | 13.26 | 13.38 | 13.51 | 13.63 | 13.76 |
| 5.1 | 12 63 | 12 75 | 12 88 | 13.00 | 13.13 | 13 25 | 13.38 | 13 50 | 13.63 | 13.76 | 13.89 |
| 5.2 | 12 75 | 12 87 | 13 00 | 13 12 | 13 25 | 13.37 | 13.50 | 13.62 | 13.75 | 13.88 | 14.01 |
| 5.3 | 12 87 | 12 99 | 13 12 | 13.24 | 13 37 | 13 49 | 13.62 | 13.74 | 13.87 | 14.00 | 14.13 |
| 5.4 | 12 99 | 13 11 | 13 24 | 13.36 | 13 49 | 13 61 | 13.74 | 13.87 | 14.00 | 14.12 | 14.25 |
| 5.5 | 13 11 | 13.23 | 13.36 | 13 48 | 13.61 | 13 73 | 13.86 | 13.99 | 14.12 | 14.24 | 14.37 |
| 5.6 | 13 23 | 13.35 | 13 48 | 13.60 | 13 73 | 13 86 | 13.98 | 14.11 | 14.24 | 14.36 | 14.49 |
| 5.7 | 13.35 | 13.47 | 13 60 | 13 72 | 13.85 | 13 98 | 14.11 | 14.23 | 14.36 | 14.48 | 14.61 |
| 5.8 | 13 47 | 13 59 | 13 72 | 13 84 | 13 97 | 14 10 | 14.23 | 14.35 | 14.48 | 14 61 | 14.74 |
| 5.9 | 13 59 | 13 71 | 13 84 | 13 97 | 14 10 | 14 22 | 14 35 | 14 47 | 14 60 | 14 73 | 14.86 |

# LXIX

## FROM FAT AND LACTOMETER READING

AMERICAN STANDARD

AT 60° F.

| 31.5 | 32.0 | 32.5 | 33.0 | 33.5 | 34.0 | 34.5 | 35.0 | 35.5 | 36.0 | 36.5 | Fat. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7.87 | 8.00 | 8.12 | 8.25 | 8.37 | 8.50 | 8.62 | 8.75 | 8.87 | 9.00 | 9.12 | 0.0 |
| 7.99 | 8.12 | 8.24 | 8.37 | 8.49 | 8.62 | 8.74 | 8.87 | 8.99 | 9.12 | 9.24 | 0.1 |
| 8.11 | 8.24 | 9.36 | 8.49 | 8.61 | 8.74 | 8.86 | 8.99 | 9.11 | 9.24 | 9.36 | 0.2 |
| 8.23 | 8.36 | 8.48 | 8.61 | 8.73 | 8.86 | 8.98 | 9.11 | 9.23 | 9.36 | 9.48 | 0.3 |
| 8.35 | 8.48 | 8.60 | 8.73 | 8.85 | 8.98 | 9.10 | 9.23 | 9.35 | 9.48 | 9.60 | 0.4 |
| 8.47 | 8.60 | 8.72 | 8.85 | 8.97 | 9.10 | 9.22 | 9.35 | 9.47 | 9.60 | 9.72 | 0.5 |
| 8.59 | 8.72 | 8.84 | 8.97 | 9.09 | 9.22 | 9.34 | 9.47 | 9.59 | 9.72 | 9.84 | 0.6 |
| 8.71 | 8.84 | 8.96 | 9.09 | 9.21 | 9.34 | 9.46 | 9.59 | 9.71 | 9.84 | 9.96 | 0.7 |
| 8.83 | 8.96 | 9.08 | 9.21 | 9.33 | 9.46 | 9.58 | 9.71 | 9.83 | 9.96 | 10.08 | 0.8 |
| 8.95 | 9.08 | 9.20 | 9.33 | 9.45 | 9.58 | 9.70 | 9.83 | 9.95 | 10.08 | 10.20 | 0.9 |
| 9.07 | 9.20 | 9.32 | 9.45 | 9.57 | 9.70 | 9.82 | 9.95 | 10.07 | 10.20 | 10.32 | 1.0 |
| 9.19 | 9.32 | 9.44 | 9.57 | 9.69 | 9.82 | 9.94 | 10.07 | 10.19 | 10.32 | 10.44 | 1.1 |
| 9.31 | 9.44 | 9.56 | 9.69 | 9.81 | 9.94 | 10.06 | 10.19 | 10.31 | 10.44 | 10.56 | 1.2 |
| 9.43 | 9.56 | 9.68 | 9.81 | 9.93 | 10.06 | 10.18 | 10.31 | 10.43 | 10.56 | 10.68 | 1.3 |
| 9.55 | 9.68 | 9.80 | 9.93 | 10.05 | 10.18 | 10.30 | 10.43 | 10.55 | 10.68 | 10.80 | 1.4 |
| 9.67 | 9.80 | 9.92 | 10.05 | 10.17 | 10.30 | 10.42 | 10.55 | 10.67 | 10.80 | 10.92 | 1.5 |
| 9.79 | 9.92 | 10.04 | 10.17 | 10.29 | 10.42 | 10.54 | 10.67 | 10.79 | 10.92 | 11.04 | 1.6 |
| 9.91 | 10.04 | 10.16 | 10.29 | 10.41 | 10.54 | 10.66 | 10.79 | 10.91 | 11.04 | 11.16 | 1.7 |
| 10 03 | 10 16 | 10.28 | 10.41 | 10.53 | 10 66 | 10.78 | 10.91 | 11.04 | 11.17 | 11.29 | 1.8 |
| 10 15 | 10 28 | 10.40 | 10 53 | 10 65 | 10 78 | 10.90 | 11.03 | 11.16 | 11.29 | 11.41 | 1.9 |
| 10 27 | 10 40 | 10.53 | 10.66 | 10.78 | 10 91 | 11.03 | 11.16 | 11.28 | 11.41 | 11.53 | 2.0 |
| 10 39 | 10 52 | 10.65 | 10.78 | 10 90 | 11 03 | 11.15 | 11.28 | 11.40 | 11.53 | 11.65 | 2.1 |
| 10 51 | 10 64 | 10.77 | 10.90 | 11.02 | 11.15 | 11.27 | 11.40 | 11.52 | 11.65 | 11.77 | 2.2 |
| 10 63 | 10.76 | 10.89 | 11.02 | 11.14 | 11 27 | 11.39 | 11.52 | 11.64 | 11.77 | 11.89 | 2.3 |
| 10 75 | 10.88 | 11.01 | 11.14 | 11.26 | 11 39 | 11.51 | 11.64 | 11.76 | 11.89 | 12.01 | 2.4 |
| 10.87 | 11.00 | 11.13 | 11.26 | 11.38 | 11 51 | 11.63 | 11.76 | 11.88 | 12.01 | 12.13 | 2.5 |
| 10.99 | 11.12 | 11.25 | 11.38 | 11.50 | 11 63 | 11.75 | 11.88 | 12.00 | 12.13 | 12.25 | 2.6 |
| 11.11 | 11 24 | 11 37 | 11.50 | 11.62 | 11 75 | 11.87 | 12.00 | 12.12 | 12.25 | 12.37 | 2.7 |
| 11.23 | 11.37 | 11.49 | 11.62 | 11.74 | 11.87 | 11.99 | 12.12 | 12.24 | 12.37 | 12.49 | 2.8 |
| 11.36 | 11.49 | 11.61 | 11.74 | 11.86 | 11.99 | 12.11 | 12.24 | 12.36 | 12.47 | 12.61 | 2.9 |
| 11.48 | 11 61 | 11.73 | 11.86 | 11.98 | 12.11 | 12.23 | 12.36 | 12.49 | 12.61 | 12.74 | 3.0 |
| 11.60 | 11 73 | 11.85 | 11.98 | 12.10 | 12.23 | 12.35 | 12.48 | 12.61 | 12.74 | 12.86 | 3.1 |
| 11.72 | 11.85 | 11.97 | 12.10 | 12.22 | 12.35 | 12.48 | 12.61 | 12.73 | 12.86 | 12.98 | 3.2 |
| 11.84 | 11.97 | 12.09 | 12.22 | 12.35 | 12.48 | 12.60 | 12.73 | 12.85 | 12.98 | 13.10 | 3.3 |
| 11.96 | 12.09 | 12.21 | 12.34 | 12.47 | 12.60 | 12.72 | 12.85 | 12.97 | 13.10 | 13.22 | 3.4 |
| 12.08 | 12.21 | 12.33 | 12.46 | 12.59 | 12.72 | 12.84 | 12.97 | 13.09 | 13.22 | 13.34 | 3.5 |
| 12.20 | 12.33 | 12.45 | 12.58 | 12.71 | 12 84 | 12.96 | 13.09 | 13.21 | 13.34 | 13.46 | 3.6 |
| 12.32 | 12.45 | 12.57 | 12.70 | 12.83 | 12.96 | 13.08 | 13.21 | 13.33 | 13.46 | 13.58 | 3.7 |
| 12 44 | 12.57 | 12.69 | 12.82 | 12.95 | 13.08 | 13.20 | 13.33 | 13.45 | 13.58 | 13.70 | 3.8 |
| 12.56 | 12.69 | 12.81 | 12.94 | 13.07 | 13.20 | 13.32 | 13.45 | 13.57 | 13.70 | 13.83 | 3.9 |
| 12.68 | 12.81 | 12 93 | 13.06 | 13.19 | 13.32 | 13.44 | 13.57 | 13.70 | 13.83 | 13.95 | 4.0 |
| 12.80 | 12.93 | 13.05 | 13.18 | 13.31 | 13.44 | 13.56 | 13.69 | 13.82 | 13.95 | 14.07 | 4.1 |
| 12.92 | 13.05 | 13.18 | 13.31 | 13.43 | 13.56 | 13.69 | 13.82 | 13.94 | 14.07 | 14.19 | 4.2 |
| 13.05 | 13.18 | 13.30 | 13.43 | 13.55 | 13.68 | 13.81 | 13.94 | 14.06 | 14.19 | 14.31 | 4.3 |
| 13.17 | 13.30 | 13.42 | 13.55 | 13.67 | 13.80 | 13.93 | 14.06 | 14.18 | 14.31 | 14.43 | 4.4 |
| 13.29 | 13.42 | 13.54 | 13.67 | 13.79 | 13.92 | 14.05 | 14.18 | 14.30 | 14.43 | 14.55 | 4.5 |
| 13.41 | 13.54 | 13 66 | 13.79 | 13.91 | 14 04 | 14.17 | 14.30 | 14.42 | 14.55 | 14.67 | 4.6 |
| 13.53 | 13.66 | 13.78 | 13.91 | 14 03 | 14.16 | 14.29 | 14.42 | 14.54 | 14.67 | 14 79 | 4.7 |
| 13.65 | 13.78 | 13.90 | 14.03 | 14.15 | 14 28 | 14.41 | 14.54 | 14.66 | 14.79 | 14.91 | 4.8 |
| 13.77 | 13.90 | 14.02 | 14.15 | 14.27 | 14.40 | 14.53 | 14.66 | 14.78 | 14.91 | 15.03 | 4.9 |
| 13.89 | 14.02 | 14.14 | 14.27 | 14.39 | 14.52 | 14.65 | 14.78 | 14.90 | 15.03 | 15.15 | 5.0 |
| 14.01 | 14.14 | 14.26 | 14.39 | 14.51 | 14.64 | 14.77 | 14.90 | 15.02 | 15.15 | 15.27 | 5.1 |
| 14.13 | 14.26 | 14 38 | 14.51 | 14 63 | 14.76 | 14 89 | 15.02 | 15.14 | 15.27 | 15.39 | 5.2 |
| 14.25 | 14.38 | 14.50 | 14.63 | 14.75 | 14.88 | 15.01 | 15.14 | 15.26 | 15.39 | 15.51 | 5.3 |
| 14.37 | 14.50 | 14.62 | 14.75 | 14.88 | 15.01 | 15.13 | 15.26 | 15.38 | 15.51 | 15.63 | 5.4 |
| 14.49 | 14.62 | 14.75 | 14.87 | 15.00 | 15.13 | 15.25 | 15.38 | 15.50 | 15.63 | 15.75 | 5.5 |
| 14.61 | 14.75 | 14.87 | 14.99 | 15.12 | 15.25 | 15.37 | 15.50 | 15.62 | 15.75 | 15 87 | 5.6 |
| 14.74 | 14.87 | 14.99 | 15.11 | 15.24 | 15.37 | 15.49 | 15.62 | 15.74 | 15.87 | 15.99 | 5.7 |
| 14.86 | 14.99 | 15.11 | 15.23 | 15.36 | 15.49 | 15.61 | 15.74 | 15 86 | 15 99 | 16.12 | 5.8 |
| 14.98 | 15.11 | 15.23 | 15.36 | 15.48 | 15.61 | 15.73 | 15.86 | 15 99 | 16.12 | 16.24 | 5.9 |

## TABLE FOR CALCULATING TOTAL SOLIDS FROM ACCORDING TO

| Fat. | LACTOMETER READING | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 26.0 | 26.5 | 27.0 | 27.5 | 28.0 | 28.5 | 29.0 | 29.5 | 30.0 | 30.5 | 31.0 |
| 0.0 | 6.652 | 6.776 | 6.900 | 7.025 | 7.150 | 7.274 | 7.397 | 7.522 | 7.647 | 7.771 | 7.895 |
| 0.1 | 6.77 | 6.90 | 7.02 | 7.15 | 7.27 | 7.39 | 7.52 | 7.64 | 7.77 | 7.89 | 8.02 |
| 0.2 | 6.89 | 7.02 | 7.14 | 7.26 | 7.39 | 7.51 | 7.64 | 7.76 | 7.89 | 8.01 | 8.14 |
| 0.3 | 7.01 | 7.14 | 7.26 | 7.39 | 7.51 | 7.63 | 7.76 | 7.88 | 8.01 | 8.13 | 8.26 |
| 0.4 | 7.13 | 7.26 | 7.38 | 7.51 | 7.63 | 7.75 | 7.88 | 8.00 | 8.13 | 8.25 | 8.38 |
| 0.5 | 7.25 | 7.38 | 7.50 | 7.63 | 7.75 | 7.87 | 8.00 | 8.12 | 8.25 | 8.37 | 8.50 |
| 0.6 | 7.37 | 7.50 | 7.62 | 7.75 | 7.87 | 7.99 | 8.12 | 8.24 | 8.37 | 8.49 | 8.62 |
| 0.7 | 7.49 | 7.62 | 7.74 | 7.87 | 7.99 | 8.11 | 8.24 | 8.36 | 8.49 | 8.61 | 8.74 |
| 0.8 | 7.61 | 7.74 | 7.86 | 7.99 | 8.11 | 8.23 | 8.36 | 8.48 | 8.61 | 8.73 | 8.86 |
| 0.9 | 7.73 | 7.86 | 7.98 | 8.11 | 8.23 | 8.35 | 8.48 | 8.60 | 8.73 | 8.85 | 8.98 |
| 1.0 | 7.85 | 7.98 | 8.10 | 8.23 | 8.35 | 8.47 | 8.60 | 8.72 | 8.85 | 8.97 | 9.10 |
| 1.1 | 7.97 | 8.10 | 8.22 | 8.35 | 8.47 | 8.59 | 8.72 | 8.84 | 8.97 | 9.09 | 9.22 |
| 1.2 | 8.09 | 8.22 | 8.34 | 8.47 | 8.59 | 8.71 | 8.84 | 8.96 | 9.09 | 9.21 | 9.34 |
| 1.3 | 8.21 | 8.34 | 8.46 | 8.59 | 8.71 | 8.83 | 8.96 | 9.08 | 9.21 | 9.33 | 9.46 |
| 1.4 | 8.33 | 8.46 | 8.58 | 8.71 | 8.83 | 8.95 | 9.08 | 9.20 | 9.33 | 9.45 | 9.58 |
| 1.5 | 8.45 | 8.58 | 8.70 | 8.83 | 8.95 | 9.07 | 9.20 | 9.32 | 9.45 | 9.57 | 9.70 |
| 1.6 | 8.57 | 8.70 | 8.82 | 8.95 | 9.07 | 9.19 | 9.32 | 9.44 | 9.57 | 9.69 | 9.82 |
| 1.7 | 8.69 | 8.82 | 8.94 | 9.07 | 9.19 | 9.31 | 9.44 | 9.56 | 9.69 | 9.81 | 9.94 |
| 1.8 | 8.81 | 8.94 | 9.06 | 9.19 | 9.31 | 9.43 | 9.56 | 9.68 | 9.81 | 9.93 | 10.06 |
| 1.9 | 8.93 | 9.06 | 9.18 | 9.31 | 9.43 | 9.55 | 9.68 | 9.80 | 9.93 | 10.05 | 10.18 |
| 2.0 | 9.05 | 9.18 | 9.30 | 9.43 | 9.55 | 9.67 | 9.80 | 9.92 | 10.05 | 10.17 | 10.30 |
| 2.1 | 9.17 | 9.30 | 9.42 | 9.55 | 9.67 | 9.79 | 9.92 | 10.04 | 10.17 | 10.29 | 10.42 |
| 2.2 | 9.29 | 9.42 | 9.54 | 9.67 | 9.79 | 9.91 | 10.04 | 10.16 | 10.29 | 10.41 | 10.54 |
| 2.3 | 9.41 | 9.54 | 9.66 | 9.79 | 9.91 | 10.03 | 10.16 | 10.28 | 10.41 | 10.53 | 10.66 |
| 2.4 | 9.53 | 9.66 | 9.78 | 9.91 | 10.03 | 10.15 | 10.28 | 10.40 | 10.53 | 10.65 | 10.78 |
| 2.5 | 9.65 | 9.78 | 9.90 | 10.03 | 10.15 | 10.27 | 10.40 | 10.52 | 10.65 | 10.77 | 10.90 |
| 2.6 | 9.77 | 9.90 | 10.02 | 10.15 | 10.27 | 10.39 | 10.52 | 10.64 | 10.77 | 10.89 | 11.02 |
| 2.7 | 9.89 | 10.02 | 10.14 | 10.27 | 10.39 | 10.51 | 10.64 | 10.76 | 10.89 | 11.01 | 11.14 |
| 2.8 | 10.01 | 10.14 | 10.26 | 10.39 | 10.51 | 10.63 | 10.76 | 10.88 | 11.01 | 11.13 | 11.26 |
| 2.9 | 10.13 | 10.26 | 10.38 | 10.51 | 10.63 | 10.75 | 10.88 | 11.00 | 11.13 | 11.25 | 11.38 |
| 3.0 | 10.25 | 10.38 | 10.50 | 10.63 | 10.75 | 10.87 | 11.00 | 11.12 | 11.25 | 11.37 | 11.50 |
| 3.1 | 10.37 | 10.50 | 10.62 | 10.75 | 10.87 | 10.99 | 11.12 | 11.24 | 11.37 | 11.49 | 11.62 |
| 3.2 | 10.49 | 10.62 | 10.74 | 10.87 | 10.99 | 11.11 | 11.24 | 11.36 | 11.49 | 11.61 | 11.74 |
| 3.3 | 10.61 | 10.74 | 10.86 | 10.99 | 11.11 | 11.23 | 11.36 | 11.48 | 11.61 | 11.73 | 11.86 |
| 3.4 | 10.73 | 10.86 | 10.98 | 11.11 | 11.23 | 11.35 | 11.48 | 11.60 | 11.73 | 11.85 | 11.98 |
| 3.5 | 10.85 | 10.98 | 11.10 | 11.23 | 11.35 | 11.47 | 11.60 | 11.72 | 11.85 | 11.97 | 12.10 |
| 3.6 | 10.97 | 11.10 | 11.22 | 11.35 | 11.47 | 11.59 | 11.72 | 11.84 | 11.97 | 12.09 | 12.22 |
| 3.7 | 11.09 | 11.22 | 11.34 | 11.47 | 11.59 | 11.71 | 11.84 | 11.96 | 12.09 | 12.21 | 12.34 |
| 3.8 | 11.21 | 11.34 | 11.46 | 11.59 | 11.71 | 11.83 | 11.96 | 12.08 | 12.21 | 12.33 | 12.46 |
| 3.9 | 11.33 | 11.46 | 11.58 | 11.71 | 11.83 | 11.95 | 12.08 | 12.20 | 12.33 | 12.45 | 12.58 |
| 4.0 | 11.45 | 11.58 | 11.70 | 11.83 | 11.95 | 12.07 | 12.20 | 12.32 | 12.45 | 12.57 | 12.70 |
| 4.1 | 11.57 | 11.70 | 11.82 | 11.95 | 12.07 | 12.19 | 12.32 | 12.44 | 12.57 | 12.69 | 12.82 |
| 4.2 | 11.69 | 11.82 | 11.94 | 12.07 | 12.19 | 12.31 | 12.44 | 12.56 | 12.69 | 12.81 | 12.94 |
| 4.3 | 11.81 | 11.94 | 12.06 | 12.19 | 12.31 | 12.43 | 12.56 | 12.68 | 12.81 | 12.93 | 13.06 |
| 4.4 | 11.93 | 12.06 | 12.18 | 12.31 | 12.43 | 12.55 | 12.68 | 12.80 | 12.93 | 13.05 | 13.18 |
| 4.5 | 12.05 | 12.18 | 12.30 | 12.43 | 12.55 | 12.67 | 12.80 | 12.92 | 13.05 | 13.17 | 13.30 |
| 4.6 | 12.17 | 12.30 | 12.42 | 12.55 | 12.67 | 12.79 | 12.92 | 13.04 | 13.17 | 13.29 | 13.42 |
| 4.7 | 12.29 | 12.42 | 12.54 | 12.67 | 12.79 | 12.91 | 13.04 | 13.16 | 13.29 | 13.41 | 13.54 |
| 4.8 | 12.41 | 12.54 | 12.66 | 12.79 | 12.91 | 13.03 | 13.16 | 13.28 | 13.41 | 13.53 | 13.66 |
| 4.9 | 12.53 | 12.66 | 12.78 | 12.91 | 13.03 | 13.15 | 13.28 | 13.40 | 13.53 | 13.65 | 13.78 |
| 5.0 | 12.65 | 12.78 | 12.90 | 13.03 | 13.15 | 13.27 | 13.40 | 13.52 | 13.65 | 13.77 | 13.90 |
| 5.1 | 12.77 | 12.90 | 13.02 | 13.15 | 13.27 | 13.39 | 13.52 | 13.64 | 13.77 | 13.89 | 14.02 |
| 5.2 | 12.89 | 13.02 | 13.14 | 13.27 | 13.39 | 13.51 | 13.64 | 13.76 | 13.89 | 14.01 | 14.14 |
| 5.3 | 13.01 | 13.14 | 13.26 | 13.39 | 13.51 | 13.63 | 13.76 | 13.88 | 14.01 | 14.13 | 14.26 |
| 5.4 | 13.13 | 13.26 | 13.38 | 13.51 | 13.63 | 13.75 | 13.88 | 14.00 | 14.13 | 14.25 | 14.38 |
| 5.5 | 13.25 | 13.38 | 13.50 | 13.63 | 13.75 | 13.87 | 14.00 | 14.12 | 14.25 | 14.37 | 14.50 |
| 5.6 | 13.37 | 13.50 | 13.62 | 13.75 | 13.87 | 13.99 | 14.12 | 14.24 | 14.37 | 14.49 | 14.62 |
| 5.7 | 13.49 | 13.62 | 13.74 | 13.87 | 13.99 | 14.11 | 14.24 | 14.36 | 14.49 | 14.61 | 14.74 |
| 5.8 | 13.61 | 13.74 | 13.86 | 13.99 | 14.11 | 14.23 | 14.36 | 14.48 | 14.61 | 14.73 | 14.86 |
| 5.9 | 13.73 | 13.86 | 13.98 | 14.11 | 14.23 | 14.35 | 14.48 | 14.60 | 14.73 | 14.85 | 14.98 |

# LXX

## FAT AND LACTOMETER READING

### Droop Richmond

| AT 60° F. | | | | | | | | | | | Fat. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 31.5 | 32 0 | 32 5 | 33 0 | 33 5 | 34 0 | 34 5 | 35.0 | 35.5 | 36.5 | 36.5 | |
| 8.018 | 8 140 | 8 264 | 8 387 | 8 509 | 8 631 | 8 755 | 8.878 | 9.000 | 9.122 | 9.244 | 0.0 |
| 8.14 | 8 26 | 8 38 | 8 51 | 8 63 | 8.75 | 8 88 | 9.00 | 9.12 | 9.24 | 9.36 | 0.1 |
| 8 26 | 8 38 | 8 50 | 8 63 | 8 75 | 8 87 | 9.00 | 9.12 | 9 24 | 9.36 | 9.48 | 0.2 |
| 8 38 | 8 50 | 8 62 | 8 75 | 8 87 | 8 99 | 9.12 | 9 24 | 9.36 | 9.48 | 9.60 | 0.3 |
| 8 50 | 8 62 | 8 74 | 8 87 | 8 99 | 9.11 | 9.24 | 9.36 | 9.48 | 9.60 | 9.72 | 0.4 |
| 8 62 | 8 74 | 8.86 | 8 99 | 9 11 | 9.23 | 9.36 | 9.48 | 9.60 | 9.72 | 9.84 | 0.5 |
| 8.74 | 8 86 | 8 98 | 9 11 | 9 23 | 9 35 | 9 48 | 9 60 | 9.72 | 9.84 | 9.96 | 0.6 |
| 8 86 | 8 98 | 9 10 | 9 23 | 9 35 | 9 47 | 9 60 | 9.72 | 9 84 | 9.96 | 10.08 | 0.7 |
| 8.98 | 9 10 | 9 22 | 9 35 | 9 47 | 9.59 | 9 72 | 9 84 | 9 96 | 10 08 | 10.20 | 0.8 |
| 9.10 | 9 22 | 9 34 | 9 47 | 9 59 | 9 71 | 9 84 | 9 96 | 10.08 | 10.20 | 10.32 | 0.9 |
| 9.22 | 9 34 | 9.46 | 9 59 | 9 71 | 9.83 | 9 96 | 10 08 | 10 20 | 10.32 | 10.44 | 1.0 |
| 9.34 | 9 46 | 9.58 | 9.71 | 9.83 | 9.95 | 10 08 | 10 20 | 10.32 | 10 44 | 10.56 | 1.1 |
| 9.46 | 9.58 | 9.70 | 9 83 | 9 95 | 10 07 | 10 20 | 10 32 | 10 44 | 10.56 | 10.68 | 1.2 |
| 9.58 | 9 70 | 9 82 | 9 95 | 10 07 | 10 19 | 10.32 | 10 44 | 10 56 | 10 68 | 10.80 | 1.3 |
| 9.70 | 9 82 | 9.94 | 10 07 | 10 19 | 10 31 | 10 44 | 10 56 | 10 68 | 10.80 | 10.92 | 1.4 |
| 9.82 | 9 94 | 10 06 | 10 19 | 10.31 | 10.43 | 10 56 | 10 68 | 10 80 | 10 92 | 11.04 | 1.5 |
| 9 94 | 10 06 | 10 18 | 10 31 | 10 43 | 10 55 | 10 68 | 10 80 | 10 92 | 11.04 | 11.16 | 1.6 |
| 10.06 | 10 18 | 10 30 | 10.43 | 10 55 | 10 67 | 10 80 | 10 92 | 11 04 | 11.16 | 11.28 | 1.7 |
| 10 18 | 10 30 | 10 42 | 10 55 | 10 67 | 10 79 | 10 92 | 11 04 | 11.16 | 11.28 | 11.40 | 1.8 |
| 10 30 | 10 42 | 10 54 | 10 67 | 10.79 | 10 91 | 11 04 | 11 16 | 11 28 | 11.40 | 11.52 | 1.9 |
| 10 42 | 10 54 | 10.66 | 10 79 | 10 91 | 11.03 | 11.16 | 11 28 | 11.40 | 11.52 | 11.64 | 2.0 |
| 10 54 | 10 66 | 10 78 | 10 91 | 11.03 | 11.15 | 11.28 | 11.40 | 11.52 | 11.64 | 11.76 | 2.1 |
| 10 66 | 10 78 | 10.90 | 11 03 | 11.15 | 11.27 | 11 40 | 11 52 | 11.64 | 11.76 | 11.88 | 2.2 |
| 10 78 | 10 90 | 11.02 | 11 15 | 11 27 | 11 39 | 11 52 | 11 64 | 11.76 | 11.88 | 12.00 | 2.3 |
| 10.90 | 11.02 | 11.14 | 11.27 | 11 39 | 11.51 | 11 64 | 11.76 | 11 88 | 12 00 | 12 12 | 2.4 |
| 11.02 | 11.14 | 11.26 | 11.39 | 11.51 | 11 63 | 11 76 | 11 88 | 12.00 | 12.12 | 12.24 | 2.5 |
| 11.14 | 11.26 | 11.38 | 11.51 | 11.63 | 11.75 | 11.88 | 12 00 | 12.12 | 12 24 | 12.36 | 2.6 |
| 11.26 | 11.38 | 11.50 | 11.63 | 11.75 | 11 87 | 12.00 | 12.12 | 12.24 | 12.36 | 12.48 | 2.7 |
| 11.38 | 11.50 | 11.62 | 11.75 | 11.87 | 11 99 | 12 12 | 12 24 | 12 36 | 12 48 | 12 60 | 2.8 |
| 11.50 | 11.62 | 11.74 | 11.87 | 11.99 | 12 11 | 12 24 | 12 36 | 12.48 | 12 60 | 12.72 | 2.9 |
| 11.62 | 11.74 | 11.86 | 11.99 | 12.11 | 12.23 | 12.36 | 12.48 | 12.60 | 12.72 | 12.84 | 3.0 |
| 11.74 | 11.86 | 11.98 | 12 11 | 12.22 | 12.35 | 12 48 | 12.60 | 12 72 | 12 84 | 12.96 | 3.1 |
| 11.86 | 11 98 | 12.10 | 12.23 | 12 35 | 12.47 | 12 60 | 12 72 | 12 84 | 12.96 | 13.08 | 3.2 |
| 11.98 | 12 10 | 12 22 | 12.35 | 12.47 | 12 59 | 12 72 | 12 84 | 12 96 | 13.08 | 13.20 | 3.3 |
| 12.10 | 12 22 | 12.34 | 12.47 | 12.59 | 12 71 | 12 84 | 12 96 | 13 08 | 13.20 | 13.32 | 3.4 |
| 12.22 | 12 34 | 12.46 | 12.59 | 12.71 | 12 83 | 12 96 | 13 08 | 13.20 | 13.32 | 13.44 | 3.5 |
| 12.34 | 12.46 | 12.58 | 12 71 | 12.83 | 12 95 | 13 08 | 13.20 | 13 32 | 13.44 | 13.56 | 3.6 |
| 12.46 | 12.58 | 12.70 | 12.83 | 12.95 | 13.07 | 13 20 | 13.32 | 13.44 | 13.56 | 13.68 | 3.7 |
| 12.58 | 12.70 | 12.82 | 12.95 | 13.07 | 13.19 | 13.32 | 13.44 | 13.56 | 13.68 | 13.80 | 3.8 |
| 12.70 | 12.82 | 12.94 | 13.07 | 13.19 | 13.31 | 13.44 | 13.56 | 13.68 | 13.80 | 13.92 | 3.9 |
| 12.82 | 12.94 | 13.06 | 13.19 | 13.31 | 13.43 | 13 56 | 13 68 | 13 80 | 13.92 | 14 04 | 4 0 |
| 12.94 | 13.06 | 13.18 | 13.31 | 13.43 | 13.55 | 13 68 | 13 80 | 13 92 | 14.04 | 14 16 | 4.1 |
| 13.06 | 13.18 | 13.30 | 13.43 | 13.55 | 13.67 | 13 80 | 13 92 | 14 04 | 14.16 | 14.28 | 4.2 |
| 13.18 | 13.30 | 13.42 | 13 55 | 13.67 | 13 79 | 13 92 | 14 04 | 14 16 | 14 28 | 14.40 | 4 3 |
| 13.30 | 13.42 | 13 54 | 13.67 | 13.79 | 13 91 | 14 04 | 14 16 | 14 28 | 14.40 | 14.52 | 4.4 |
| 13.42 | 13 54 | 13.66 | 13.79 | 13.91 | 14 02 | 14 16 | 14.28 | 14 40 | 14.52 | 14.64 | 4.5 |
| 13.54 | 13 66 | 13.78 | 13 91 | 14 03 | 14.15 | 14 28 | 14.40 | 14.52 | 14.64 | 14.76 | 4.6 |
| 13.66 | 13.78 | 13 90 | 14.03 | 14.15 | 14.27 | 14 40 | 14.52 | 14.64 | 14.76 | 14.88 | 4.7 |
| 13.78 | 13.90 | 14.02 | 14.15 | 14.27 | 14.39 | 14.52 | 14.64 | 14 76 | 14.88 | 15.00 | 4 8 |
| 13.90 | 14.02 | 14.14 | 14.27 | 14.39 | 14.51 | 14 64 | 14.76 | 14.88 | 15.00 | 15.12 | 4.9 |
| 14.02 | 14.14 | 14.26 | 14.39 | 14 51 | 14 63 | 14 76 | 14 88 | 15.00 | 15 12 | 15.24 | 5.0 |
| 14.14 | 14.26 | 14.38 | 14.51 | 14.63 | 14.75 | 14 88 | 15.00 | 15 12 | 15.24 | 15.36 | 5 1 |
| 14.26 | 14.38 | 14.50 | 14.63 | 14 75 | 14.87 | 15 00 | 15 12 | 15.24 | 15.36 | 15 48 | 5.2 |
| 14.38 | 14.50 | 14.62 | 14.75 | 14.87 | 14.99 | 15.12 | 15.24 | 15.36 | 15.48 | 15.60 | 5 3 |
| 14.50 | 14.62 | 14.74 | 14.87 | 14.99 | 15.11 | 15.24 | 15.36 | 15.48 | 15.60 | 15.72 | 5.4 |
| 14.62 | 14.74 | 14.86 | 14.99 | 15.11 | 15.23 | 15.36 | 15 48 | 15.60 | 15.72 | 15.84 | 5.5 |
| 14.74 | 14.86 | 14.98 | 15.11 | 15.23 | 15.35 | 15.48 | 15.60 | 15 72 | 15.84 | 15.96 | 5.6 |
| 14.86 | 14.98 | 15.10 | 15.23 | 15.35 | 15.47 | 15.60 | 15.72 | 15.84 | 15.96 | 16.08 | 5.7 |
| 14.98 | 15.10 | 15.22 | 15.35 | 15.47 | 15.59 | 15.72 | 15.84 | 15.96 | 16.08 | 16.20 | 5.8 |
| 15.10 | 15.22 | 15.34 | 15 47 | 15.59 | 15.71 | 15.84 | 15.96 | 16.08 | 16.20 | 16.32 | 5.9 |

## Table LXXI

### TABLE FOR CONVERSION OF CUPROUS OXIDE ($Cu_2O$) AND COPPER TO LACTOSE

Milligrams

| $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose |
|---|---|---|---|---|---|---|---|---|
| 112.6 | 100 | 71.6 | 157.6 | 140 | 101.3 | 202.7 | 180 | 131.6 |
| 113.7 | 101 | 72.4 | 158.7 | 141 | 102.0 | 203.8 | 181 | 132.4 |
| 114.8 | 102 | 73.1 | 159.8 | 142 | 102.8 | 204.9 | 182 | 133.1 |
| 115.9 | 103 | 73.8 | 160.9 | 143 | 103.5 | 206.0 | 183 | 133.9 |
| 117.0 | 104 | 74.6 | 162.0 | 144 | 104.3 | 207.1 | 184 | 134.7 |
| 118.2 | 105 | 75.3 | 163.2 | 145 | 105.1 | 208.3 | 185 | 135.4 |
| 119.3 | 106 | 76.1 | 164.3 | 146 | 105.8 | 209.4 | 186 | 136.2 |
| 120.4 | 107 | 76.8 | 165.5 | 147 | 106.6 | 210.5 | 187 | 137.0 |
| 121.5 | 108 | 77.6 | 166.6 | 148 | 107.3 | 211.6 | 188 | 137.7 |
| 122.7 | 109 | 78.3 | 167.7 | 149 | 108.1 | 212.7 | 189 | 138.5 |
| 123.8 | 110 | 79.0 | 168.9 | 150 | 108.8 | 213.9 | 190 | 139.3 |
| 124.9 | 111 | 79.8 | 170.0 | 151 | 109.6 | 215.0 | 191 | 140.0 |
| 126.0 | 112 | 80.5 | 171.1 | 152 | 110.3 | 216.1 | 192 | 140.8 |
| 127.1 | 113 | 81.3 | 172.2 | 153 | 111.1 | 217.2 | 193 | 141.6 |
| 128.2 | 114 | 82.0 | 173.3 | 154 | 111.9 | 218.3 | 194 | 142.3 |
| 129.4 | 115 | 82.7 | 174.5 | 155 | 112.6 | 219.5 | 195 | 143.1 |
| 130.5 | 116 | 83.5 | 175.6 | 156 | 113.4 | 220.6 | 196 | 143.9 |
| 131.7 | 117 | 84.2 | 176.7 | 157 | 114.1 | 221.8 | 197 | 144.6 |
| 132.8 | 118 | 85.0 | 177.8 | 158 | 114.9 | 222.9 | 198 | 145.4 |
| 133.9 | 119 | 85.7 | 178.9 | 159 | 115.6 | 224.0 | 199 | 146.2 |
| 135.1 | 120 | 86.4 | 180.1 | 160 | 116.4 | 225.2 | 200 | 146.9 |
| 136.2 | 121 | 87.2 | 181.2 | 161 | 117.1 | 226.3 | 201 | 147.7 |
| 137.3 | 122 | 87.9 | 182.3 | 162 | 117.9 | 227.4 | 202 | 148.5 |
| 138.4 | 123 | 88.7 | 183.4 | 163 | 118.6 | 228.5 | 203 | 149.2 |
| 139.5 | 124 | 89.4 | 184.5 | 164 | 119.4 | 229.6 | 204 | 150.0 |
| 140.7 | 125 | 90.1 | 185.7 | 165 | 120.2 | 230.7 | 205 | 150.7 |
| 141.8 | 126 | 90.9 | 186.8 | 166 | 120.9 | 231.9 | 206 | 151.5 |
| 143.0 | 127 | 91.6 | 188.0 | 167 | 121.7 | 233.0 | 207 | 152.2 |
| 144.1 | 128 | 92.4 | 189.1 | 168 | 122.4 | 234.1 | 208 | 153.0 |
| 145.2 | 129 | 93.1 | 190.2 | 169 | 123.2 | 235.2 | 209 | 153.7 |
| 146.4 | 130 | 93.8 | 191.4 | 170 | 123.9 | 236.4 | 210 | 154.5 |
| 147.5 | 131 | 94.6 | 192.5 | 171 | 124.7 | 237.5 | 211 | 155.2 |
| 148.6 | 132 | 95.3 | 193.6 | 172 | 125.5 | 238.6 | 212 | 156.0 |
| 149.7 | 133 | 96.1 | 194.7 | 173 | 126.2 | 239.7 | 213 | 156.7 |
| 150.8 | 134 | 96.9 | 195.8 | 174 | 127.0 | 240.8 | 214 | 157.5 |
| 152.9 | 135 | 97.6 | 197.0 | 175 | 127.8 | 242.0 | 215 | 158.2 |
| 153.1 | 136 | 98.3 | 198.1 | 176 | 128.5 | 243.1 | 216 | 159.0 |
| 154.2 | 137 | 99.1 | 199.3 | 177 | 129.3 | 244.3 | 217 | 159.7 |
| 155.3 | 138 | 99.8 | 200.4 | 178 | 130.1 | 245.4 | 218 | 160.4 |
| 156.4 | 139 | 100.5 | 201.5 | 179 | 130.8 | 246.5 | 219 | 161.2 |

TABLE LXXI—*Continued*

TABLE FOR CONVERSION OF CUPROUS OXIDE ($Cu_2O$) AND COPPER TO LACTOSE

MILLIGRAMS

| $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose |
|---|---|---|---|---|---|---|---|---|
| 247.7 | 220 | 161.9 | 292.7 | 260 | 192.5 | 337.8 | 300 | 224.4 |
| 248.8 | 221 | 162.7 | 293.8 | 261 | 193.3 | 338.9 | 301 | 225.2 |
| 249.9 | 222 | 163.4 | 294.9 | 262 | 194.1 | 340.0 | 302 | 225.9 |
| 251.0 | 223 | 164.2 | 296.0 | 263 | 194.9 | 341.1 | 303 | 226.7 |
| 252.1 | 224 | 164.9 | 297.1 | 264 | 195.7 | 342.2 | 304 | 227.5 |
| 253.3 | 225 | 165.7 | 298.3 | 265 | 196.4 | 343.4 | 305 | 228.3 |
| 254.4 | 226 | 166.4 | 299.4 | 266 | 197.2 | 344.5 | 306 | 229.1 |
| 255.5 | 227 | 167.2 | 300.5 | 267 | 198.0 | 345.6 | 307 | 229.8 |
| 256.6 | 228 | 167.9 | 301.6 | 268 | 198.8 | 346.7 | 308 | 230.6 |
| 257.7 | 229 | 168.6 | 302.7 | 269 | 199.5 | 347.8 | 309 | 231.4 |
| 258.9 | 230 | 169.4 | 303.9 | 270 | 200.3 | 349.0 | 310 | 232.2 |
| 260.0 | 231 | 170.1 | 305.0 | 271 | 201.1 | 350.1 | 311 | 232.9 |
| 261.1 | 232 | 170.9 | 306.2 | 272 | 201.9 | 351.2 | 312 | 233.7 |
| 262.2 | 233 | 171.6 | 307.3 | 273 | 202.7 | 352.3 | 313 | 234.5 |
| 263.3 | 234 | 172.4 | 308.4 | 274 | 203.5 | 353.4 | 314 | 235.3 |
| 264.5 | 235 | 173.1 | 309.6 | 275 | 204.3 | 354.6 | 315 | 236.1 |
| 265.6 | 236 | 173.9 | 310.7 | 276 | 205.1 | 355.7 | 316 | 236.8 |
| 266.8 | 237 | 174.6 | 311.8 | 277 | 205.9 | 356.8 | 317 | 237.6 |
| 267.9 | 238 | 175.4 | 313.0 | 278 | 206.7 | 357.9 | 318 | 238.4 |
| 269.0 | 239 | 176.2 | 314.1 | 279 | 207.5 | 359.0 | 319 | 239.2 |
| 270.2 | 240 | 176.9 | 315.3 | 280 | 208.3 | 360.2 | 320 | 240.0 |
| 271.3 | 241 | 177.7 | 316.4 | 281 | 209.1 | 361.3 | 321 | 240.7 |
| 272.4 | 242 | 178.5 | 317.5 | 282 | 209.9 | 362.4 | 322 | 241.5 |
| 273.5 | 243 | 179.3 | 318.6 | 283 | 210.7 | 363.5 | 323 | 242.3 |
| 274.6 | 244 | 180.1 | 319.7 | 284 | 211.5 | 364.6 | 324 | 243.1 |
| 275.8 | 245 | 180.8 | 320.9 | 285 | 212.3 | 365.8 | 325 | 243.9 |
| 276.9 | 246 | 181.6 | 322.0 | 286 | 213.1 | 366.9 | 326 | 244.6 |
| 278.1 | 247 | 182.4 | 323.1 | 287 | 213.9 | 368.0 | 327 | 245.4 |
| 279.2 | 248 | 183.2 | 324.2 | 288 | 214.7 | 369.1 | 328 | 246.2 |
| 280.3 | 249 | 184.0 | 325.3 | 289 | 215.5 | 370.2 | 329 | 247.0 |
| 281.5 | 250 | 184.4 | 326.5 | 290 | 216.3 | 371.4 | 330 | 247.7 |
| 282.6 | 251 | 185.5 | 327.6 | 291 | 217.1 | 372.5 | 331 | 248.5 |
| 283.7 | 252 | 186.3 | 328.7 | 292 | 217.9 | 373.6 | 332 | 249.2 |
| 284.8 | 253 | 187.1 | 329.8 | 293 | 218.7 | 374.7 | 333 | 250.0 |
| 286.0 | 254 | 187.9 | 330.9 | 294 | 219.5 | 375.8 | 334 | 250.8 |
| 287.1 | 255 | 188.7 | 332.1 | 295 | 220.3 | 377.0 | 335 | 251.6 |
| 288.2 | 256 | 189.4 | 333.2 | 296 | 221.1 | 378.1 | 336 | 252.5 |
| 289.3 | 257 | 190.2 | 334.4 | 297 | 221.9 | 379.3 | 337 | 253.3 |
| 290.4 | 258 | 191.0 | 335.5 | 298 | 222.7 | 380.4 | 338 | 254.1 |
| 291.5 | 259 | 191.8 | 336.7 | 299 | 223.5 | 381.5 | 339 | 254.9 |

## TABLE LXXI—*Continued*

## TABLE FOR CONVERSION OF CUPROUS OXIDE ($Cu_2O$) AND COPPER TO LACTOSE

MILLIGRAMS

| $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose | $Cu_2O$ | Cu | Lactose |
|---|---|---|---|---|---|---|---|---|
| 382.7 | 340 | 255.7 | 405.3 | 360 | 272.1 | 427.9 | 380 | 289.1 |
| 383.8 | 341 | 256.5 | 406.4 | 361 | 272.9 | 429.0 | 381 | 289.9 |
| 385.0 | 342 | 257.4 | 407.5 | 362 | 273.7 | 430.1 | 382 | 290.8 |
| 386.1 | 343 | 258.2 | 408.6 | 363 | 274.5 | 431.2 | 383 | 291.7 |
| 387.2 | 344 | 259.0 | 409.7 | 364 | 275.3 | 432.3 | 384 | 292.5 |
| 388.4 | 345 | 259.8 | 410.9 | 365 | 276.2 | 433.5 | 385 | 293.4 |
| 389.5 | 346 | 260.6 | 412.0 | 366 | 277.1 | 434.6 | 386 | 294.2 |
| 390.6 | 347 | 261.4 | 413.1 | 367 | 277.9 | 435.8 | 387 | 295.1 |
| 391.7 | 348 | 262.3 | 414.2 | 368 | 278.8 | 436.9 | 388 | 296.0 |
| 392.8 | 349 | 263.1 | 415.3 | 369 | 279.6 | 438.0 | 389 | 296.8 |
| 394.0 | 350 | 263.9 | 416.5 | 370 | 280.5 | 439.2 | 390 | 297.7 |
| 395.1 | 351 | 264.7 | 417.6 | 371 | 281.4 | 440.3 | 391 | 298.5 |
| 396.2 | 352 | 265.5 | 418.8 | 372 | 282.2 | 441.4 | 392 | 299.4 |
| 397.3 | 353 | 266.3 | 419.9 | 373 | 283.1 | 442.5 | 393 | 300.3 |
| 398.4 | 354 | 267.2 | 421.0 | 374 | 283.9 | 443.6 | 394 | 301.1 |
| 399.6 | 355 | 268.0 | 422.2 | 375 | 284.8 | 444.8 | 395 | 302.0 |
| 400.7 | 356 | 268.8 | 423.3 | 376 | 285.7 | 445.9 | 396 | 302.8 |
| 401.9 | 357 | 269.6 | 424.5 | 377 | 286.5 | 447.0 | 397 | 303.7 |
| 403.0 | 358 | 270.4 | 425.6 | 378 | 287.4 | 448.1 | 398 | 304.6 |
| 404.1 | 359 | 271.2 | 426.7 | 379 | 288.2 | 449.2 | 399 | 305.4 |
| | | | | | | 450.4 | 400 | 306.3 |

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